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Some Effects of Carcinogenic Agents on Mice Subject to Spontaneous Leukoses*†

John J. Morton, M.D., and G. Burroughs Mider, M.D.‡

(From the Department of Surgery, The University of Rochester School of Medicine and Dentistry, Rochester, New York)

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During the past 2 years we have been investigating the effect of painting with methylcholanthrene on the development of leukosis in dilute brown (strain Dba) mice (4, 5). The strain has been extensively studied by Little and Murray (3, 6, 7). Although it is generally considered to have a high incidence of spontaneous mammary carcinoma, several sublines have been developed in which the tumor incidence varies. Line 212 has a relatively high incidence of spontaneous "lymphoblastoma" or lymphomatosis while in line 122 breast tumors predominate. Pulmonary tumors are rarely found. The exact incidence of spontaneous nonepithelial tumors is not known. They occur less frequently than in the C57 black mice. In the latter strain, investigated at the Roscoe B. Jackson Memorial Laboratory, 13.79 per cent of 877 mice developed nonepithelial tumors when 700 to 750 days old (3).

Spontaneous lymphomatosis appears in dilute brown mice at an age of 650 to 800 days at the Jackson Laboratory. It is found in the dilute brown stock at the New York State Institute for the Study of Malignant Disease in mice more than 500 days old. The leukosis is characterized grossly by splenomegaly and enlargement of 1 or more groups of lymph nodes. In the spontaneous disease the mesenteric, retroperitoneal, and mediastinal nodes are involved more frequently than the cervical, axillary, or inguinal groups. The microscopic picture is that of leukemic infiltration of organs and tissues with cells resembling lymphoblasts.

The original experiments were performed on a group of 50 dilute brown mice obtained from the Jackson Laboratory. They represented a mixture of various lines of the strain. They were painted twice weekly until death with a 0.5 per cent solution of methylcholanthrene in benzene at 9 successive sites. Three different types of leukosis were produced in 77 per cent of the group (Table I).

The most frequent lesion (75.5 per cent) was a general lymphomatosis in which most of the lymph nodes of the body were enlarged. Splenomegaly and hepatomegaly were present. Anasarca occurred as a terminal event. The cell population in the affected tissue was similar to that seen in the spontaneous disease. The abnormal cells replaced the lymph nodes and bone marrow completely and infiltrated liver, spleen, intestine, kidneys, bladder, genitalia, pancreas, skin, muscle, and subcutaneous tissues. Hyperleucocytosis from 50,000 to 300,000 was present. Transplantation of affected tissues to mice of the same strain reproduced the widespread adenopathy and infiltrations of the original disease.

TABLE I: LEUKOSIS IN DILUTE BROWN MICE

Agent	Number of mice	General lymphomatosis	Mediastinal lymphoma	Reticulo-endotheliosis	Total leukoses
Methylcholanthrene 0.5% in benzene	48	28	8	1	37
3,4-Benzpyrene 0.5% in benzene	48	20	10	0	30
Benzene	50	10	1	0	11

Solitary mediastinal lymphomas were found in 21.5 per cent of the mice that developed leukosis. Other than a large mediastinal mass, no gross tumor was demonstrable. Abnormal cells that appeared to be identical with those previously described were found only in the mediastinal tumor. The bone marrow, lymph nodes, and blood stream were not involved.

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‡ Research Fellow, The National Cancer Institute, National Institute of Health, U. S. Public Health Service, Bethesda, Maryland.

Transplantation of 3 of these tumors to 30 other dilute brown mice failed.

The 3rd type of lesion was a pathological curiosity which has been termed reticulo-endotheliosis. We have observed only 1 case. The gross features resembled those of general lymphomatosis. The type of cell that characterized this condition was large, acidophilic, and frequently multinucleated. It appeared to be actively phagocytic. The distribution was that seen in general lymphomatosis. The hematopoietic elements of the bone marrow were depleted.

A similar group of dilute brown mice was painted in the same manner with a 0.5 per cent solution of 3,4-benzpyrene in benzene. Only 62.5 per cent of them developed leukosis. General lymphomatosis comprised 66.6 per cent. Half of these showed no gross lymphadenopathy although the characteristic type of cell was present in the usual distribution. The variation following benzpyrene resembled that found in the spontaneous disease. This might have been due to the difference in chemical structure, the diminution in the total stimulus, or to substrain differences in the mice that were painted.

Fifty more dilute brown mice were painted with benzene alone. This experiment has been in progress for more than 540 days. Six mice are still alive and appear healthy. Ten of the 44 that have died had a modified form of general lymphomatosis. Generalized lymphadenopathy was found in 2 of these. In the other 8 only abdominal lymph nodes were involved. Infiltration of liver, spleen, lung, and kidneys was noted, yet the bone marrow showed little or no change. The mean age at which the lymphomatosis was found, 496.3 days, leads one to question whether benzene alone had any pronounced influence on the latent period [1] of the spontaneous disease.

These preliminary observations established that some carcinogenic agents could be made to influence the latent period of leukosis in dilute brown mice. A breeding colony of line 212 stock was obtained from the Jackson Laboratory. This facilitated study of the disease since the lineage of each animal obtained by brother-sister mating was known. It is unfortunate that adequate controls are lacking. We have not had a single case of spontaneous leukosis in any of our breeding colony to date. Thirty of them have lived more than 500 days. Mammary carcinomas, primary lung tumors, and a sarcoma of the uterus have been found.

One hundred fifty-six identified line 212 dilute brown mice survived painting with a 0.5 per cent solution of methylcholanthrene in benzene for more than 60 days. The incidence of leukosis in these animals was 98.7 per cent. Two died without tumor, 150 had general lymphomatosis, and 4, mediastinal lymphoma. The sex did not influence the reaction. The incidence and latent period of leukosis were almost identical for males and females (Table II).

TABLE II: EFFECT OF SEX ON INDUCED LEUKOSIS IN LINE 212 DILUTE BROWN MICE

	Males	Females	Total
Incidence, per cent	98.7	98.6	98.7
Mean latent period, days	86.1 ± 1.4	90.0 ± 1.4	87.7 ± 0.9
Standard deviation	13.4	11.1	12.4
Variation	15.5%	12.3%	13.7%

The effect of the age of the mouse at the time that painting was commenced has been investigated (Table III). Little variation in the latent period was found before the mice were 80 days old. In the following age groups, 81-100 and 100+ days, the latent period was prolonged. Further experiments with older mice have been commenced. One might expect that painting older mice would hasten the appearance of the disease. The increase in time observed suggests that the animals developed some degree of resistance either to the action of methylcholanthrene or to the tumor cells. Although this question cannot be decided at present, the following evidence may be cited.

TABLE III: EFFECT OF AGE ON LATENT PERIOD OF INDUCED LEUKOSIS IN LINE 212 DILUTE BROWN MICE

Age in days when painting started	Number of mice	Latent period, in days
1-20	8	89.6
21-40	41	86.7
41-60	31	86.2
61-80	45	91.9
81-100	11	95.8
101+	14	99.5
Total	150	87.8

The age of the mouse within 60 to 160 days did not influence the latent period or growth rate of transplanted lymphomatosis. If the prolonged latent interval in older mice was due to relative increase in resistance to the neoplasm, then the period from onset to death should be longer. This factor is subject to considerable variation but no significant change in relation to age could be demonstrated.

In the subsequent experiments the line 212 mice were divided into identified litter mate groups. One of these groups was painted with a 0.5 per cent solution of methylcholanthrene in benzene, the other with the chemical or solvent under investigation (Table IV). The incidence of leukosis was not

[1] Latent period refers to the time elapsed between the first painting with methylcholanthrene solution and the appearance of detectable lymphadenopathy.

altered, but the latent period was lengthened when a 0.25 per cent solution of methylcholanthrene in benzene was used. An equal concentration of methylcholanthrene in acetone resulted in approximately the same latent period as did the benzene solution, but the incidence of leukosis was reduced. More mediastinal lymphomas occurred than in any previous group. Five of the 22 females that were painted developed breast tumors without evidence of lymphomatosis. Breast carcinomas were present in groups painted with carcinogenic agents in benzene solution also. These, however, were associated with general lymphomatosis. If the mice with mammary tumors were added to those with leukosis, the tumor incidence, 92.8 per cent, approached that found for the other groups painted with methylcholanthrene. Acetone solutions of methylcholanthrene might have a different action than benzene solutions. Benzene affects the hematopoietic system. When already damaged it might be more susceptible to the action of methylcholanthrene than one that remains relatively normal when painted with acetone. This problem is receiving further consideration at present.

TABLE IV: LEUKOSIS IN LINE 212 DILUTE BROWN MICE

Agent	Number of mice	General lymphomatosis: Number	General lymphomatosis: Latent period, in days	Mediastinal lymphoma: Number	Mediastinal lymphoma: Latent period, in days	Per cent leukosis
Methylcholanthrene 0.5% in benzene	156	150	87.8	4	91.7	98.7
Methylcholanthrene 0.25% in benzene	44	41	100.1	2	89.0	97.7
Methylcholanthrene 0.25% in acetone	53	38	103.1	5	114.6	81.6
3,4-Benzpyrene 0.5% in benzene	49	38	126.4	1	113.0	79.5

Control . . . . . No leukosis in 30 mice living more than 450 days.

The experience with a 0.5 per cent solution of 3,4-benzpyrene in benzene bore out the differences between the action of methylcholanthrene and benzpyrene described in the literature. The latent period was prolonged and the incidence of leukosis was decreased. The 10 mice that did not develop leukosis lived at least 122 days and the mean duration of painting them was 156 days. The general lymphomatosis that followed painting with benzpyrene was essentially the same as that induced by methylcholanthrene. The variation noted in the unidentified group of dilute brown mice must have been due to a mixture of different substrains, probably a difference in the genetic pattern of the ancestors.

Identified dilute brown mice of line 122 (high mammary carcinoma) were painted in the same manner with a 0.5 per cent solution of methylcholanthrene in benzene. Characteristic general lymphomatosis developed in each of the 12 mice that were painted. The latent period was 122 days. No breast tumors were obtained.

Little is known concerning the reaction of the blood leucocytes to painting with methylcholanthrene in benzene. The mice with fully developed lymphomatosis had a leucocytosis exceeding 100,000 cells per cu. mm. More than 90 per cent of them were immature cells resembling lymphoblasts. Serial leucocyte counts indicated that the characteristic abnormal cells appeared in the circulating blood 2 to 4 weeks before lymphadenopathy could be recognized. Their numbers were small at first but increased rapidly. The neutrophilic elements became depleted before the lymphocytes did. Some of the mice showed evidence of an anemia. Megaloblasts and normoblasts appeared in the peripheral blood. In a mouse that developed a breast tumor without leukosis in the course of painting the leucocytes rose rapidly. This was due to an increase in adult segmented neutrophiles. Myeloblasts and myelocytes were rarely seen. The organs of this mouse did not show evidence of leukemic infiltration. The bone marrow was hyperplastic but showed no evidence of a maturation defect.

Five other strains of mice were painted with a 0.5 per cent solution of methylcholanthrene in benzene in the same manner as were the dilute browns. C albino, C3H and C57 black strains were obtained from the Roscoe B. Jackson Memorial Laboratory, and the old and new Buffalo strains were from the Biological Station of the New York State Institute for the Study of Malignant Diseases. All of the mice were between 4 and 6 weeks old when painting was started. The results indicated that the occurrence of spontaneous leukosis in a mouse strain does not mean that painting with methylcholanthrene will increase the incidence or decrease the latent interval of production (Table V). The incidence of spontaneous leukemia is relatively high in C albino and C57 black stock, yet no cases were obtained after the administration of methylcholanthrene. The incidence of induced leukosis was low in the other 3 strains. Again, adequate controls are lacking so that no comparison with the spontaneous rate may be made. Leukoses followed painting with methylcholanthrene only in those strains of mice that had a high incidence of spontaneous mammary carcinoma.

The leukosis produced in old Buffalo, new Buffalo, and C3H mice by methylcholanthrene was morphologically identical with that seen in the dilute brown strain. The spontaneous disease affecting the C57 black

family appears to be the same also. While we have termed them lymphomatoses this designation is open to criticism. The identification of immature mouse leucocytes by staining reactions is fallible. Morphologic studies are inconclusive and facilities for studying the characteristic cells *in vitro* have been lacking. Such an error in interpretation of the fundamentally affected cell would account for the bizarre results.

None of the mice that developed a primary lung tumor had concurrent lymphomatosis. Death from pulmonary neoplasm cannot be the sole reason why these animals did not develop leukosis. The latent period for each of the tumors was approximately the same. Skin tumors, epidermoid carcinomas, and subcutaneous sarcomas, usually occurred somewhat earlier than did leukosis. It is possible that they killed the mice before a leukosis had an opportunity to appear. The presence of concurrent skin and lung tumors or skin tumors and leukosis was infrequent.

TABLE V: EFFECT OF METHYLCHOLANTHRENE 0.5 PER CENT IN BENZENE ON 5 STRAINS OF MICE

Strain	Number painted	Days painted	Leukosis	Tumors			No tumor
				Skin	Lung	Breast	
Old Buffalo	37	125	2	21	18	0	9
New Buffalo	29	125	5	14	13	0	8
C3H	46	174	5	38	11	1	5
C albino	42	174	0	21	16	0	14
C57 black	34	250	0	8	0	0	26

In the C albino and C57 black strains nonmalignant extramedullary myelopoiesis was a common autopsy finding. This lesion, described by Lewis (2), and by Barnes and Sisman (1), was characterized by enlargement of the liver and spleen. The lymph nodes were not grossly altered. The histologic appearance of the organs was similar to that seen in myeloid leukosis. Evidence of erythropoiesis and a preponderance of adult neutrophiles readily distinguished the lesion from a true leukosis. The bone marrow was hyperplastic but evidence of maturation was seen. The heterotopic cells which characterized the methylcholanthrene leukosis were not encountered. The condition could not be transmitted to mice of the same strain by subcutaneous transplantation of affected organs or tissues. Extramedullary myelopoiesis was also found in dilute brown mice that were painted with benzene alone.

SUMMARY AND CONCLUSIONS

The appearance of leukosis in dilute brown mice may be hastened by painting them with: (a) a 0.5 per cent solution of methylcholanthrene in benzene, (b) a 0.25 per cent solution of methylcholanthrene in benzene, (c) a 0.25 per cent solution of methylcholanthrene in acetone, or, (d) a 0.5 per cent solution of 3,4-benzpyrene in benzene. Painting with benzene alone does not have an effect of the same magnitude. The incidence of leukosis in line 212 of the dilute brown strain is greater after painting with methylcholanthrene than after painting with benzpyrene. Preliminary reports of the alterations in the blood leucocytes that precede the disease are presented.

Leukosis was found in old Buffalo, new Buffalo, and C3H mice after painting with a 0.5 per cent solution of methylcholanthrene in benzene but not in the C57 black or C albino strains. All of these mice are known to develop spontaneous leukosis although the incidence is not established.

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The Effect of Nursing on the Incidence of Spontaneous Leukemia and Tumors in Mice*

W. A. Barnes, M.D., and R. K. Cole, Ph.D.

(From the Department of Pathology, Cornell University Medical College, New York, N. Y., and the Department of Poultry Husbandry, Cornell University, Ithaca, N. Y.)

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This preliminary study was undertaken to determine if nonchromosomal factors similar to those which influence the occurrence of breast tumors of mice (1, 3, 5, 8, 9) exist in relation to leukemia.

MacDowell and Richter (7) presented evidence that nonchromosomal factors may influence the development of leukemia in mice. Of the F_1 hybrids whose mothers were from the leukemic stock C58, 61.9 per cent developed leukemia, while the disease occurred in only 42.5 per cent of hybrids with mothers from the relatively nonleukemic stock Sto Li. In backcrosses of F_1 hybrids (with mothers from the leukemic stock) to the relatively nonleukemic stock, the incidence of leukemia was greater among those offspring whose hybrid parent was the mother (46.5 per cent) than among those whose father was the hybrid (19.8 per cent). In nursing experiments, of which no details are presented, these authors found no increase in the incidence of leukemia in mice of a relatively nonleukemic stock (Sto Li) fostered by mothers of a leukemic stock (C58) and no reduction in the occurrence of this disease in mice of a leukemic stock (C58) nursed by mice of stocks with low incidence of leukemia.

The present study is based on over 600 fostered mice observed from birth until natural death. The mice used in these experiments were from approximately the 16th to the 24th generations of 3 stocks that have been inbred in this laboratory. Their origin has been previously described (4). The incidences of spontaneous leukemias and tumors in these stocks from the 16th to the 24th generation are indicated in Table I.

Table I shows that the incidence of leukemia is low in stocks Rf and Af, and high in stock Ak. Breast tumors are common in both virgin and bred mice of stock Af, while in stock Rf lung tumors are numerous, but there are few neoplasms of other types. In the fostering experiments, the Ak mice were from lines Akf and Akh in approximately equal numbers.

Expectant mothers were separated individually and their cages examined early in the morning and late in the afternoon every day. Litters were exchanged within 12 hours of delivery and a record made of the number of offspring. Because of the possibility of incomplete delivery, subsequent records were made of the number of babies in the cage, 1 and 7 days after exchange. The mice were observed until their natural death. Gross post-mortem examination was supplemented by microscopic examination whenever necessary. Only mice living at least 7 months were analyzed, unless otherwise stated.

TABLE I: INCIDENCE OF LEUKEMIAS AND TUMORS FROM 16TH TO 24TH GENERATIONS OF 3 STOCKS OF MICE

Stock of mice	Breast tumor per cent	Lung tumor per cent	Leukemia per cent
Ak (432 mice)	0	0	60.0
Line Akf (126 mice)	0	0	43.0
Line Akh (186 mice)	0	0	76.0
Line Aki (120 mice)	0	0	54.0
Rf ♀ virgin (53 mice)	0	25	
♀ breeding (49 mice)	0	40	4.0
♂ (99 mice)	0	35	
Af ♀ virgin (128 mice)	41		
♀ breeding (60 mice)	50	4.4	0.5
♂ (121 mice)	0		

RESULTS OF NURSING EXPERIMENTS

Leukemia.—Figure 1 shows the relation of age to the incidence of leukemia in nonfostered mice of the leukemic stock Ak and of those fostered by mice of the nonleukemic stocks Af or Rf. Of 176 fostered mice that survived 6 months, 57 developed leukemia, an incidence of 32 per cent. This is considerably lower than that observed among nonfostered mice of stock Ak (60.0 per cent). This suggests the presence of extrachromosomal factors for resistance to leukemia. However, mortality due to disease other than leukemia (mainly pneumonia) was high among fostered mice between 9 and 15 months of age, and further study is required to determine whether this apparent decrease in the incidence of leukemia among fostered mice is due to a nursing factor. In experiments to be reported elsewhere, numerous reciprocal matings of hybrid mice of stocks with high and low incidence of leukemia indicate that the occurrence of leukemia is affected by nonchromosomal maternal factors.

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In the reciprocal test, 181 mice of the nonleukemic stocks were nursed by females of the leukemic stock Ak. Only 4 of these mice died with leukemia, approximately 2 per cent, which is not higher than the percentage of leukemia in nonfostered mice of these stocks. This indicates that leukemia was not produced by nursing mice of these stocks having a low incidence of leukemia, by mice of our highly leukemic stock.

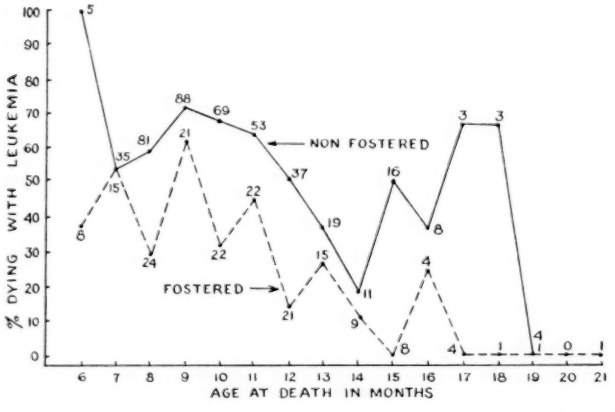

FIG. 1.—Incidence of leukemia among nonfostered mice of stock Ak compared with that among mice of this stock fostered by mice of stocks Af and Rf with a low incidence of leukemia. The total number of mice dead each month is indicated.

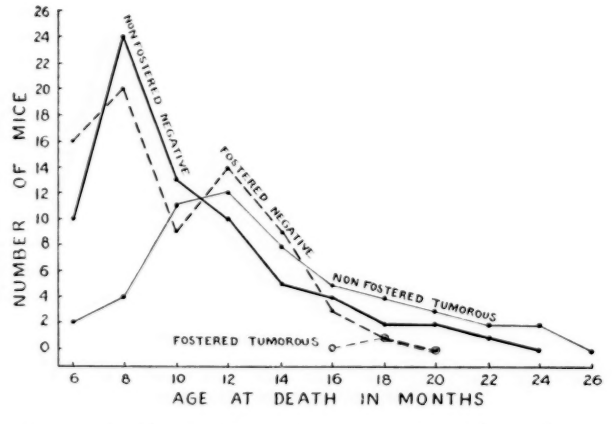

FIG. 2.—Incidence of breast tumors among nonfostered virgin female mice of a stock (Af) with a high incidence of breast tumors, compared with that among virgin females fostered by mice of stocks having very few breast tumors.

Breast tumors.—Mice of stock Af, among which breast tumors are common, were used in these studies as controls. Although breeding increases the occurrence of breast tumors in this stock only slightly, the virgin females alone will be considered. Figure 2 shows that of 63 virgin female mice of stock Af nursed by mothers of a stock with a low incidence of breast tumors (Ak or Rf), only 1 developed a mammary tumor, a percentage of 1.6, as compared with 41 per cent in virgin female mice nursed by their own mothers. The chart indicates that the decrease in the incidence of breast tumors under the conditions of the experiment is significant.

In the reciprocal test made by fostering mice of stocks without mammary carcinoma (Ak and Rf) by females of stock Af, with a high incidence of mammary cancer, none of the 82 female mice, only 4 of which had offspring, developed breast tumors. Breast tumors occur in stock Af at a relatively late age, with a majority of cases occurring between 10 and 15 months, and many mice of stock Ak died, usually with leukemia, before this age. Although 58 fostered mice of this series lived 12 months or longer, further study is necessary to determine if mice of stock Af possess extrachromosomal factors transmissible by nursing, which influence the development of breast cancer (3).

TABLE II: COMPARISON OF INCIDENCE OF LUNG TUMORS AMONG NONFOSTERED MICE OF STOCK RF AND AMONG THOSE FOSTERED BY FEMALES OF STOCKS WITH LOW INCIDENCE OF LUNG TUMORS (AF AND AK)

	Total number of mice	Number of mice with lung tumors	Per cent with lung tumors
♂, nonfostered	99	35	35
♂, fostered	58	10	18
♀, virgin, nonfostered	53	13	25
♀, virgin, fostered	59	7	11
♀, bred, nonfostered	49	20	40
♂ and virgin ♀, nonfostered	152	48	31.6
♂ and virgin ♀, fostered	117	17	14.5

Lung tumors.—Table II shows the incidence of primary lung tumors among nonfostered mice of a stock (Rf) with a high incidence of this neoplasm, and among Rf mice fostered by females of stocks having very few lung tumors (Af and Ak). There is apparently a conspicuous difference between fostered and nonfostered mice. An analysis by the χ^2 test gives the following figures:

	No. lung tumors	Lung tumor	No. of mice	
♂, nonfostered	64	35	99	$\chi^2 = 5.8677$
♂, fostered	48	10	58	$P = < .02$
Total	112	45	157	
♀, virgin, nonfostered	40	13	53	$\chi^2 = 3.0525$
♀, virgin, fostered	52	7	59	$P = > .05$
Total	92	20	112	

When the data are combined, the χ^2 values added, and the table of probability entered at 2 degrees of freedom, the P value is found to be $<.02$.

Thus, as a result of fostering, there seems to be a significant reduction in the incidence of lung tumors in the males, a questionable decrease among the virgin females and a significant reduction when the data for the sexes are combined. However, fostered mice died

at an earlier age than nonfostered animals. The highest death rate among fostered mice was between 12 and 14 months. In nonfostered mice lung tumors were more common in older mice. This nullifies the apparent difference in the incidence of primary lung tumors between fostered and nonfostered mice. The reverse experiment failed to show nonchromosomal factors in relation to lung tumors, the incidence of these tumors in 98 mice of stocks Ak and Af fostered by mice of stock Rf remaining low (3 per cent). While the number of mice in fostered and control mice studies is small, the data indicate that fostering does not markedly influence the incidence of lung tumors, as already observed by others (6).

Further studies are in progress in attempts to clarify some of the findings here described.

Summary and Conclusions

Mice of the stocks studied having a low incidence of leukemia were not rendered leukemic by nursing with mice of a stock (Ak) having a high incidence of leukemia. Mice of a highly leukemic stock (Ak) fostered by mice of relatively nonleukemic stocks (Rf and Af) developed leukemia in lower percentage than their nonfostered relatives. Intercurrent death was slightly higher among fostered than among nonfostered mice and this may account in part for the lower incidence of the disease among fostered mice.

Mice of stock Af having a high incidence of breast tumors failed to develop tumors, with a single exception, when nursed by mice of stocks having a very few breast tumors. Breast tumors did not develop in mice of these stocks with a low incidence of breast tumors when nursed by Af mice.

There are 3 factors that may influence the occurrence of breast cancer in mice (2); *i.e.,* (a) a genetic factor for susceptibility, transmitted probably as a dominant complex; (b) a hormonal influence, with differences in the susceptibility to estrogenic hormones apparently dependent on the strain; (c) an "extrachromosomal (milk) factor", the nature of which is as yet undetermined.

If mice of stock Af carry extrachromosomal factors responsible for the development of breast tumors, these may be weaker or present in lower concentration than those described by others (1, 3, 5, 8, 9). It is also possible that mice of our stocks with a low incidence of breast tumors are resistant to the extrachromosomal influence (*i.e.,* lack a genetic tendency for susceptibility to breast tumors), but in recent, as yet incomplete, experiments, breast tumors have occurred in 4 Ak mice nursed by females of stock C3H (stock of Little, with high incidence of breast tumors).

In the stocks studied, the incidence of lung tumors was not significantly altered by fostering.

Mr. Julius Mesiar assisted in this work.

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Acceleration of the Development of Leukemias and Mammary Carcinomas in Mice by 9,10-Dimethyl-1,2-Benzanthracene*

J. Engelbreth-Holm and H. Lefèvre

(From the Cancer Research Laboratory of The Danish Anti-Cancer League, Copenhagen, Denmark)

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During comparative investigations into the reaction of different inbred strains of mice to treatment with carcinogenic hydrocarbons, it appeared in some experiments that leukemias or tumor formations occurred in greater numbers and earlier in the treated animals of strains in which tumors or leukemia develop spontaneously than in untreated animals of similar strains. An acceleration of the kinds of tumors occurring in these strains was evidently involved.

An account will be given in the present paper of experiments with 9,10-dimethyl-1,2-benzanthracene administered to 2 strains of mice, a mammary carcinoma strain, dilute brown, and a leukemic strain, Aka.

Material and Technic

Mice of 2 strains were used. One of these was Little's dilute brown, Dlb, among the untreated animals of which mammary carcinoma develops spontaneously in about 50 per cent of the females over 9 months old and leukemia in about 2 per cent of both males and females over 8 months old. The other strain was Furth's Aka, in which from 50 to 70 per cent of the animals that survive to the age of 8 months die of leukemia, usually lymphogenous leukemia.

Four to 8 months old mice of these strains were treated with 9,10-dimethyl-1,2-benzanthracene (prepared by Professor Haakon Lund, Aarhus, Denmark), the administration being in some cases by subcutaneous injection of from 0.5 to 1 mgm. dissolved in sterile olive oil and in others by painting the skin of the back 2 or 3 times a week with 0.5 per cent solution in benzene.

The substance employed is fairly toxic and the resistance of animals of different strains varies. Eighteen out of 20 Dlb mice died within 2 months of the subcutaneous injection of 1 mgm. The same dose was tolerated better by strain Aka, of which 8 out of 20 died in the course of 3 months. Similarly, painting every other day with 0.5 per cent of the solution caused a great loss of test animals, especially among the Dlb mice, whereas painting every 3rd day was tolerated quite well.

Experiments

Altogether 44 dilute brown mice were given from 0.5 to 1 mgm. 9,10-dimethyl-1,2-benzanthracene by subcutaneous injection. Twenty-five of these animals died either from intercurrent diseases, pneumonia or enteritis, or from the toxic action of the injection. Before the surviving 19 animals were 8 months old, 4 cases of leukemia (2 ♀, 2 ♂), 1 case of lymphosarcoma (♀), 3 cases of mammary carcinoma, and 1 case of ovarian carcinoma (all ♀) developed. No case of tumorous development or leukemia was observed in the control animals, siblings to the test animals, before they were 8 months old. In addition to these tumors, which were of the same types as those seen spontaneously in this strain, spindle cell sarcomas developed in 7 cases at the site of the injection. Five of these were observed in animals in which no other tumor was seen to develop and 2 were found simultaneously with a lymphosarcoma and a mammary carcinoma, respectively. The results of the experiment appear in Tables I and II.

Painting 50 animals of the same strain 2 or 3 times a week with the same substance produced the following results (Tables I and III): Seventeen of the animals died from intercurrent diseases or from toxic effects. Among the 33 surviving animals (16 ♀ and 17 ♂), there were 6 cases of lymphogenous leukemia (4 ♂, 2 ♀), 1 case (♀), of myelogenous leukemia, and 7 cases of mammary carcinoma (all ♀). Three of these 7 had 2 mammary carcinomas. Painting produced tumors in 24 of these 33 animals, 23 carcinomas and 1 sarcoma of the skin. Of these, 2 cases of cancer of the skin occurred in animals with leukemia, 4 in animals with mammary carcinoma; and 17 carcinomas of the skin and 1 sarcoma of the skin were observed in animals in which neither leukemia nor mammary carcinoma developed. No case of simultaneous leukemia and mammary carcinoma was observed.

* This investigation was aided by a grant from The Lady Tata Memorial Trust.

Twenty mice of leukemic strain Aka were given subcutaneous injections of 1 mgm. 9,10-dimethyl-1,2-benzanthracene. A part of this series of experiments has been published previously (6). Eight of these animals died from intercurrent diseases or from intoxication. Among the 12 survivors leukemic changes developed in 9, and these animals died at ages varying from 15 to 21 weeks. An ovarian carcinoma was found in 1 animal. Five developed spindle cell sarcomas and 2 developed carcinoma of the skin at the site of the injection. Two of the sarcomas were found in animals without leukemia, 2 in animals with leukemia, and 1 in a mouse with ovarian carcinoma. One of the 2 carcinomas appeared in an animal with reticular cell hyperplasia in the lymphnodes. The results are summarized in Tables I and IV.

TABLE I: RESULTS OF TREATING MICE WITH 9,10-DIMETHYL-1,2-BENZANTHRACENE

	9,10-Dimethyl-1,2-benzanthracene	No. of animals treated	No. of animals surviving	No. developing leukemia or lymphosarcoma before 8 months	No. developing mammary carcinoma before 9 months	No. developing malignant tumors at site of injection
Strain Aka	1 mgm. injected subcutaneously	20	12	9	0	7
	0.5% solution in benzene applied cutaneously	14	11	10	0	7
	Total	34	23	19	0	14
	Untreated controls	40	40	0	0	..
Strain Dlb	0.5-1 mgm. injected subcutaneously	44	19	5	3	7
	0.5% solution in benzene cutaneously 2-3 times a week	50	33	7	7 *	24
	Total	94	52	12	10	31
	Untreated controls	40	36	0	2	.

* Three of these mice had multiple mammary carcinomas.

TABLE II: RESULTS OF TREATING DILUTE BROWN MICE WITH 0.5 MGM. 9,10-DIMETHYL-1,2-BENZANTHRACENE INJECTED SUBCUTANEOUSLY

Mouse no.	Age at death, in weeks	Changes at site of injection	Other findings
188 ♀	21	Lymphosarcoma 15x15x5 mm. large	All lymphnodes large, 3-5 mm. Spleen large, 29x9x8 mm., variegated, dark red. Liver, kidneys, thymus not enlarged. Lymphosarcomatosis in lymphnodes and spleen, with hemorrhages. Leukemic infiltration in liver.
190 ♀	15	None	Mammary carcinoma near left foreleg, 12x12 mm. A 3x3 mm. node in left lung. Many small abscesses in the kidneys.
390 ♀	29	Fusocellular sarcoma 26x20x4 mm	Typical mammary adenocarcinoma 15x10x10 mm. in perineum.
393 ♀	18	None	Stem-cell leukemia. Most lymphnodes 8-10 mm. in size. Mesenteric lymphnode 25 mm. Spleen and liver enlarged. Thymus normal. Blood: 69,000 leucocytes/c.mm. with 91.5% stem-cells/c.mm. myeloblasts.
411 ♀	36	None	Mammary adenocarcinoma, 19x17x10 mm., in left axilla.
412 ♀	24	None	Leukemia. Many lymphnodes 10 mm. Mesenteric lymphnode 19 mm. Spleen large, thymus 10x9 mm. Liver and kidneys moderately enlarged. Blood: 181,500 leucocytes/c.mm. with 97.5% stem-cells, mostly myeloblasts.
418 ♀	22	None	Leukemia. Many lymphnodes 9-15 mm. Mesenteric lymphnode 19 mm. Thymus 10x6 mm. Spleen large, reddish blue. Liver and kidneys not enlarged. Blood leukemic.
424 ♀	26	Fibrosarcoma 17x17x12 mm	Mesenteric lymphnode 10x3 mm., blood-red. Right ovary 9 mm., whitish-yellow, dark red in parts. Uterus distended, filled with blood. Ovarian tumor resembling granulosa cell type. Hyperplasia of lymphnodes.

The following results were obtained by painting the skin of the back of 14 Aka mice 3 times a week with a 0.5 per cent solution of 9,10-dimethyl-1,2-benzanthracene: Three died from intercurrent diseases; 10 of the 11 survivors died within 20 weeks with more or less pronounced leukemic or lymphosarcomatous changes,

and 5 died with carcinoma at the site of injection. The results appear in Tables I and V.

No case of spontaneous leukemia was observed in the 40 untreated control animals up to the age of 8 months during the period of the experiment.

Whereas no case of leukemia and only 2 cases of mammary carcinoma occurred during the experimental period of 9 months among 36 untreated control animals of Dlb strain, 12 cases of leukemia or lymphosarcoma and 10 of mammary carcinoma, 3 of which

TABLE III: RESULTS OF PAINTING DILUTE BROWN MICE WITH 0.5 PER CENT SOLUTION OF 9,10-DIMETHYL-1,2-BENZANTHRACENE IN BENZENE 2 OR 3 TIMES A WEEK

Mouse no.	Age at death, in weeks	Changes at site of injection	Other findings
216 ♀	31	Papillomas	2 mammary carcinomas in left axilla and left inguinal region, 15x12x10 mm. and 10x10x5 mm. Adenocarcinomas with metaplasia to squamous cell epithelium.
229 ♀	23	Papillomas	Lymphogenous leukemia. Lymphnodes 5-9 mm. Spleen large, 20x7 mm. Liver, kidneys, and thymus not enlarged. Leukemic infiltration in all organs.
256 ♀	22	Epilation	Lymphogenous leukemia. Lymphnodes 10 mm. Spleen 25x9 mm. Liver moderately enlarged. Thymus normal. Blood: 13,600 leucocytes with 92% large, atypical lymphocytes. Typical lymphogenous leukemia.
257 ♀	31	Papilloma; carcinoma	Mammary adenocarcinoma, 10x10x10 mm., on right side of neck.
261 ♂	21	Papilloma	Lymphogenous leukemia. All lymphnodes enlarged 2-5 mm. Spleen 20x5 mm. Liver enlarged. Thymus normal. Blood: 9,300 leucocytes, 87% atypical lymphocytes. Lymphatic leukemic changes in spleen and lymphnodes.
262 ♀	29	Papilloma	Mammary adenocarcinoma, 25x20x25 mm., in right side. In left axilla a subcutaneous squamous cell carcinoma, 5x5 mm.
263 ♀	32	Squamous cell carcinoma	Mammary adenocarcinoma, 10x10x10 mm., in left side.
267 ♀	24	Papilloma	Mammary adenocarcinoma, 30x25x15 mm., in left inguinal region.
268 ♀	29	Squamous cell carcinoma	Mammary adenocarcinoma, 15x15x10 mm., in left axilla.
270 ♀	22	Epilation	Myelogenous leukemia. Many lymphnodes 6-10 mm. Spleen 22x6 mm. Mesenteric lymphnode 22 mm. Liver and kidneys slightly enlarged. Thymus moderately enlarged. Blood: 71,900 leucocytes, 90% myeloblasts, 4-5% myelocytes. Leukemic infiltration in all organs.
272 ♀	25	Carcinoma and leukemic infiltrations	Lymphogenous leukemia (stem-cell ?). Many lymphnodes 6-7 mm. Mesenteric lymphnode 12 mm. Spleen 22x10 mm. Liver and kidneys slightly enlarged. Thymus normal. Blood: 41,000 leucocytes, 95.5% "blasts" (lymphogenous ?). Leukemic infiltrates in liver and lymphnodes. Severe lymphosarcoma-like infiltration subcutaneously at the site of painting.
277 ♀	34	Squamous cell carcinoma	2 mammary adenocarcinomas, 15x15x10 and 10x10x15 mm., in left side and right inguinal region. Adenocarcinomas with patches of metaplasia to squamous cell epithelium.
308 ♂	34	Squamous cell carcinoma	Lymphogenous leukemia. Many lymphnodes 5 mm. Mesenteric lymphnode 25 mm. Spleen 20x10 mm. Liver greatly enlarged. Kidneys moderately enlarged. Thymus normal. Great leukemic infiltrations in liver, kidneys, spleen, and lymphnodes. Large lymphosarcomatous infiltrations in stroma of skin carcinoma and subcutaneous tissue.
310 ♂	38	Squamous cell carcinoma	Leukemia. Lymphnodes not enlarged. Spleen 30x10 mm. Liver enlarged. Severe leukemic changes in spleen and liver with fairly large (myelogenous ?) cells. Hemorrhages in the spleen.

DISCUSSION

The use of 9,10-dimethyl-1,2-benzanthracene in painting the skin of the back and for subcutaneous injection produced local tumors at the site of treatment in 22 out of 52 Dlb and 19 out of 23 Aka mice, both inbred strains. It also caused the accelerated appearance of the tumorous diseases characteristic of these strains.

were multiple cancers, appeared among 52 animals treated. The relatively slight tendency of this strain towards the development of leukemia was thus accelerated to a pronounced degree by the treatment, the number of cases being very greatly increased, from about 2 per cent in the untreated animals to about 23 per cent. The disease developed far earlier in the lifetime of the animals than is usual, for the leukemic

animals died at ages of from 4 to 7 months, whereas spontaneous leukemia does not develop in animals of this strain until they are over the age of 8 months.

It was also possible to accelerate the development of mammary carcinoma in this strain. Its incidence does not seem to have been increased, but the tumors developed far earlier in the animals treated than in those not treated. Mammary carcinoma developed earlier than is usual in this strain in 10 out of 26 females, most had any sign of leukemia. The animals died when they were from 4 to 7 months old, whereas spontaneous leukemia does not usually develop until after the age of 9 or 10 months. The number of cases of disease also seemed to be increased to about 82 per cent, although the figures are too small for a definite conclusion to be drawn.

Of particular interest is the fact that pronounced lymphosarcomatous infiltration was observed in the

TABLE IV: RESULTS OF TREATING AKA MICE WITH 1 MGM. 9,10-DIMETHYL-1,2-BENZANTHRACENE INJECTED SUBCUTANEOUSLY

Mouse no.	Age at death, in weeks	Changes at site of injection	Other findings
2444 ♂	20	Epilation	Lymphosarcoma, 10x10 mm. in left inguinal region. Lymphnodes 5 mm. Mesenteric lymphnode 20 mm. A lymphosarcoma 30x10 mm. around right kidney. Thymus slightly enlarged. Blood: 32,100, leucocytes 96% atypical lymphocytes. Lymphogenous leukemic infiltration in liver, bone marrow, and lymphnodes.
2447 ♀	21	Reticulo-sarcoma 15x15 mm	Spleen and lymphnodes moderately enlarged. Reticulo-sarcomatosis in spleen, liver, lymphnodes. Blood normal.
2450 ♀	29	Polymorphocellular sarcoma 38x22x20 mm.	Solid carcinoma of right ovary, 10x10x10 mm., granulosa cell type. No sign of leukemia. Liver and marrow normal.
2451 ♀		Epilation. Ulceration	Lymphogenous leukemia. Lymphnodes 6-10 mm. 15x10 mm. lymphosarcoma at upper pole of right kidney. Liver, spleen, thymus only slightly enlarged. Blood: lymphogenous leukemia. Lymphogenous leukemic infiltration in lymphnodes. Liver normal.
2453 ♀	19	Epilation	Lymphosarcoma of the axilla, 10x5x3 mm. Organs otherwise normal. Blood normal.
2454 ♀	20	Squamous cell carcinoma with ulceration.	Reticular cell hyperplasia in spleen and lymphnodes. Blood: anemia, 9,600 leucocytes, 96% lymphocytes.
2456 ♂	20	Fusocellular sarcoma	Atypical lymphogenous leukemia. Lymphnodes 5 mm. Spleen 20x6 mm. Thymus slightly enlarged. Pronounced leukemic changes in liver. Blood: 46,800 leucocytes, 90% atypical lymphocytes.
2458 ♀	18	Lymphosarcoma 15x7 mm	Many lymphnodes, lumbar and inguinal, enlarged, 5 mm. Lymphosarcomatosis with extensive invasive growth into the surrounding tissues. Blood normal. A squamous cell carcinoma 18x18x14 mm. in perineum.
2460 ♂	15	Fusocellular sarcoma 5x3x2 mm	Lymphosarcoma. Lumbar lymphnodes 5 mm. Thymus slightly enlarged. Lymphosarcoma with invasion into diaphragm.
2463 ♀	19	None	Leukemic lymphosarcomatosis. Lymphnodes in neck 15x10x10 mm. Other peripheral lymphnodes 4 mm. Mesenteric lymphnode 20x7 mm. Lumbar lymphnode 13x5 mm. Thymus 10x10 mm. Blood: 45,500 leucocytes, 85% atypical lymphocytes. Bone marrow: lymphogenous leukemic. Lymphnodes: lymphosarcoma with extensive invasion of the musculature.

of the animals dying from their carcinomas at the age of 7 or 8 months, whereas spontaneous mammary cancer rarely develops before the age of 9 months. Acceleration of the development of tumors was also evident when 3 of the 10 animals died with double mammary carcinoma which, although occurring spontaneously, is far more rare. When mice of strain Aka, in which spontaneous leukemia develops in from 50 to 70 per cent of all animals, were treated with 9,10-dimethyl-1,2-benzanthracene, accelerated development of leukemic conditions occurred in 19 out of 23 animals at a time when none of the 40 control animals subcutaneous tissue at the site of painting in the cases of 5 of 11 mice of this strain that had been painted with a solution of carcinogenic hydrocarbons; and this applies both to animals in which papillomas had developed and to those in which the painting had produced carcinoma. No other sign of hyperplasia or malignant growth in the lymphatic system was found in 3 of these 5 animals, whereas general leukemic changes as well as skin infiltration was found in 2 cases.

This fact seems to indicate that in strain Aka the cells of the lymphatic system have a particular ten-

dency to react to carcinogenic influence by developing manifest malignant tumors, and that this inherited tendency applies to the whole of the lymphatic system. The development of the tumor began in these 5 cases at the sites of the painting, which were selected purely fortuitously. The same conditions are observed in strain Dlb, in which 2 of the 7 animals developed leukemia after they had been painted (Table III).

Kreyberg (10) and Cramer (4) painted mice of a high mammary carcinoma strain with tar, but observed no acceleration of the development of cancer. Nor did Bonzer and Connal (2) find any variation in the incidence of mammary carcinoma in animals painted with tar. Dobrovolskaïa-Zadvadskaïa and Adamova (5) treated mice of a strain bearing 50 per cent mammary carcinoma with dibenzanthracene or radon, but did not observe any increase in the number of mammary cancers. On the other hand, Maisin and Coolen (13) reported that they had obtained an increase in the number of mammary cancers

TABLE V: RESULTS OF PAINTING AKA MICE WITH 0.5 PER CENT SOLUTION OF 9,10-DIMETHYL-1,2-BENZANTHRACENE 3 TIMES A WEEK

Mouse no.	Age at death, in weeks	Changes at site of injection	Other findings
5031 ♀	15	Papilloma	Lymphosarcoma in the underlying subcutaneous tissue.
5032 ♀	17	Squamous cell carcinoma	Leukemic lymphosarcomatosis. All lymphnodes 3-5 mm. Widespread infiltration along the spine. Liver: lymphogenous leukemia. Lymphosarcomatous changes in lymphnodes, in infiltrations around the spine, and in the skin under painting-tumor. Blood leukemic.
5033 ♂	19	Epilation	Thymus is enlarged 7x9 mm., otherwise normal conditions. Nonleukemic hyperplasia of thymus. Blood normal.
5035 ♀	17	Squamous cell carcinoma	Killed because of paralysis of hind legs. Thymus very large, 20x15 mm. Thymus: leukemic changes with invasion of heart. Spine: severe lymphatic infiltrations epidurally and in musculature. Marrow: lymphogenous leukemia. Blood normal. Spleen and liver normal.
5036 ♀	17	Squamous cell carcinoma	Myelogenous leukemia. Peripheral lymphnodes 4-5 mm. large. The spleen somewhat enlarged. Blood: leucocytes not increased, 12.5% myeloblasts, 2.5% myelocytes. Spleen and lymphnodes consist almost entirely of myeloid tissue with large myelocytes and myeloblasts. Invasive growth around lymphnodes.
5038 ♀	18	Papillomas	Leukemic lymphosarcomatosis. A 15x10x10 mm. large lymphosarcoma in the submaxillary region. All peripheral glands 8-15 mm. Liver and spleen enlarged, thymus slightly enlarged. Blood: 170,000 leucocytes, 97% atypical lymphocytes and lymphoblasts. Liver, kidneys, pronounced lymphogenous leukemic infiltrations. All lymphnodes: lymphosarcomatous infiltration with ingrowth into the surroundings.
5039 ♀	15	Papillomas; incipient carcinoma. Lymphosarcomatosis.	Lymphosarcomatosis. Killed because of paralysis of hind legs. All lymphnodes are enlarged. Fairly extensive infiltrations in the musculature along the spine. Leukemic changes in the lymphnodes. Enormous lymphosarcomatous infiltration in and around the spine.
5040 ♀	20	Squamous cell carcinoma. Lymphosarcomatous infiltration in skin.	Subleukemic lymphosarcomatosis. Most lymphnodes enlarged, 5 mm. Mesenteric lymphnode 20x10 mm. Blood: 17,800 leucocytes, 87.5% atypical lymphocytes. Lymphosarcoma in skin under painting-tumor. Lymphnodes: lymphogenous leukemic.
5042 ♀	13	Papillomas	Severe lymphosarcomatous infiltration in subcutaneous tissue and musculature under the papillomas. Organs normal.
5043 ♀	18	Papillomas	Massive lymphocytic infiltration in subcutaneous tissue. No signs of leukemia in organs.

No antagonism was observed between the occurrence of the painting tumor and the development of mammary carcinoma or leukemia. The action of carcinogenic substances or tar on mice of inbred strains has been investigated repeatedly, but it was only recently that attention was directed to the fact that, in addition to the production of tumors at the site of painting, acceleration of the tumors characteristic of the strain can be attained.

by painting with methylcholanthrene or benzpyrene. Pronounced acceleration of the development of mammary carcinoma has been obtained in a number of experiments with estrogenic substances, by Lacassagne (11) and others. That a similar acceleration can also be obtained by painting with carcinogenic hydrocarbons appears from the experiments reported in this paper and, more particularly, from the results of another experiment in which painting with methylcholanthrene was employed (7).

The accelerated occurrence and increased incidence of leukemia or lymphosarcoma after carcinogenic action have been observed

repeatedly in strains with low and with high incidence of spontaneous leukemia.

Lacassagne (11) and Perry and Ginzton (18) found that treatment with estrogenic substances increased the incidence of leukemia or lymphosarcoma in the strains they used. Krebs, Rask-Nielsen, and Wagner (9) found 19 cases of leukemia among about 5,000 animals of a non-inbred stock treated with x-rays, whereas they found only 3 cases among 10,500 untreated animals. Furth and Furth (8) irradiated mice of an inbred strain and found that the incidence of leukemia increased seven or eightfold in 775 test animals, as compared with 1,290 control animals. Finally, convincing experiments have been published in which treatment with carcinogenic hydrocarbons has caused considerable acceleration of the cases of leukemia in the strain concerned.

Morton and Mider (15, 16) investigated dilute brown, Dba, mice of a strain in which from 80 per cent to 90 per cent of the breeding females developed mammary carcinoma at a mean age of 10.6 months. It is stated that lymphoblastoma occurs commonly in both sexes between 650 and 800 days. After painting with methylcholanthrene, 0.5 per cent solution in benzene, once a week at various sites, thus avoiding local tumors, leukemia appeared in 48 out of 60 animals at a far younger age, all the animals dying within 226 days. The earliest case was observed on the 69th day after a total of 10 paintings.

Brues and Marble (3) attained very similar results by painting with tar Bagg albino mice, in 2 per cent of which leukemia occurs spontaneously. Various kinds of tar were tested and it appeared that the occurrence of the cases of leukemia was proportional to the carcinogenic properties of the tar employed, as expressed by the number of papillomas appearing at the site of painting and by the time that lapsed before these papillomas appeared. Lymphogenous leukemia was obtained in 20 out of 40 mice painted with the most active carcinogenic tar. It is worthy of note that mice of another strain, Little's C57 black, reacted to the same treatment with the development of papillomas only, leukemia developing in no instance.

The appearance of leukemic diseases and of mammary carcinoma in animals of inbred strains can thus be accelerated when the animals are submitted to carcinogenic influences. Treatment with x-rays, estrogenic substances, or carcinogenic hydrocarbons has brought about an accelerated development of leukemia, and an accelerated development of mammary carcinoma has been observed after treatment with estrogenic substances and carcinogenic hydrocarbons. As has been mentioned, however, acceleration is not always observed when animals of tumor-bearing strains are treated with carcinogenic substances; and we still do not know what factors actually determine such acceleration.

Dosage undoubtedly plays a part in carcinogenic influence. An experiment with Aka mice, in which the animals were painted with a 0.5 per cent solution of 9,10-dimethyl-1,2-benzanthracene in benzene only twice a week and with a smaller quantity of the solution, was carried out simultaneously with that mentioned above. Under these conditions only 2 cases of accelerated leukemia were found in 38 mice, against 10 cases in 11 mice in the experiment previously mentioned.

In the experiments that have been published up to the present, reports have been given of the acceleration of tumors or leukemia that occur spontaneously in the strains concerned, while animals of tumor-free strains have not reacted in a similar manner according to the reports of Brues and Marble (3) and Lacassagne (12).

It is certainly not yet clear how the acceleration observed is to be interpreted. Recent research with mice of inbred strains has clearly shown that intrinsic inherited factors cooperate with extrinsic non-inherited factors when spontaneous tumors and leukemia are developed. It is obviously simple to regard the acceleration demonstrated in the experiment reported above as the consequence of an artificial increase of the extrinsic factors which, in conjunction with the genetic tendency of the animals to have tumors, results in earlier and more frequent manifestation of tumors. The fact that tumor-free strains react differently from tumor strains may indicate that even excessive exogenous influences cannot force the production of a tumor in an organism without a suitable genetic foundation.

Numerous experiments have shown that various exogenous influences, each in itself carcinogenic, can "potentiate" each other's influence by simultaneous action. For example, this has been shown to occur in the cases of dibenzanthracene and estrone, by Perry and Ginzton (18); irradiation with x-rays and carcinogenic hydrocarbons, by Mayneord and Parsons (14); and irradiation with x-rays and benzpyrene, by Mottram (17). It is not yet clear whether such features are analogous with the "potentiation" seen in the simultaneous action of tumor-producing virus and other carcinogenic influence, as in the experiments of Rous and Kidd (19, 20), with tar and Shope fibroma virus. Finally, how the cooperation between the genetic tendency to tumors and exogenous carcinogenic influence, observed in the experiments reported here, is to be related to the above-mentioned "potentiating" influence of two different exogenous influences cannot be decided at present.

Summary and Conclusions

1. Reports are given of the results of experiments with 9,10-dimethyl-1,2-benzanthracene administered to 2 inbred strains of mice, a strain with high incidence of mammary carcinoma and low incidence of leukemia, Dlb, and a strain with high incidence of leukemia, Aka.

2. Pronounced acceleration of the tumors characteristic for the strains, evident in increased incidence and especially in distinctly earlier appearance of the particular tumor, has been produced in these strains of mice by both subcutaneous injection of, and painting with 9,10-dimethyl-1,2-benzanthracene.

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Acceleration of the Development of Mammary Carcinomas in Mice by Methylcholanthrene*

J. Engelbreth-Holm

(From the Cancer Research Laboratory of the Danish Anti-Cancer League, Copenhagen, Denmark)

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An account was given in the previous article (7) of a series of experiments in which 9,10-dimethyl-1,2-benzanthracene, painted on the skin or subcutaneously injected, assisted or accelerated the development of leukemia not only in an inbred strain of mice, Aka, in which leukemia develops spontaneously in a large number of the animals that are over 8 months old, but also in Little's dilute brown strain, in which spontaneous leukemia occurs rarely, with an incidence of about 2 per cent. In the latter strain accelerated development was also observed in the case of mammary carcinoma, which occurs spontaneously in about 50 per cent of the females above the age of 9 months. It was doubtful, however, in this case whether the treatment had actually increased the incidence of mammary carcinoma, though there was no doubt that the animals treated developed breast cancer considerably earlier than those not treated.

Meanwhile, in another series of experiments, an undoubted acceleration of mammary carcinoma was attained, as was evident both from the increased number of tumors and from their earlier appearance.

Material and Technic

Mice of Little's dilute brown strain were used, and in this strain, as already stated, mammary carcinoma develops spontaneously in about 50 per cent of breeding females over 9 months old. Most of the tumors did not develop before the age of 12 months. In a few cases, about 2 per cent, leukemia developed spontaneously in both male and female animals that were over 6 months old.

Animals from 4 to 6 weeks old were painted once a week with a 0.5 per cent solution of methylcholanthrene in benzene in a manner similar to that employed by Murphy and Sturm (15) and by Morton and Mider (13), the substance being applied at different places so as to avoid, to some extent, the development of painting tumors in the skin. The painting was continued for 23 weeks, after which the animals were given no further treatment. At this point some of the animals had already developed mammary carcinoma, although others did not develop it until later, a period of freedom thus existing between the cessation of the treatment and the development of the tumor. In the cases of 2 animals this interval lasted for as long as 2 months.

Results

Twenty-seven females and 26 males were treated as described above. During the experimental period 8 females and 2 males died from intercurrent diseases, mostly enteritis or pneumonia.

All the remaining 19 females died from mammary carcinoma, 17 of them with multiple carcinomas. Those that survived longest were 10½ months old. One of the animals, No. 458, also had incipient lymphogenous leukemia when it died. This, in view of its low age, only 6 months, may also, presumably, be considered as due to the treatment. The changes in the individual animals are tabulated in Table I.

Table II shows the ages at death of these 19 animals compared with 19 animals, from the same generations of the strain as those from which the test animals originated, dying with mammary carcinoma.

It appears from Table II that the treatment with methylcholanthrene caused a very considerable acceleration of the tumorous development, which became manifest in the animals treated far earlier than is usual in this strain. Additional evidence of this acceleration was the fact that all the 19 females that survived the first 5 months of the experiment developed mammary carcinoma, while only 50 per cent of the untreated animals of this strain did so. Finally, the accelerated development was shown by the occurrence of more than 1 mammary carcinoma in each of 17 of the 19 test animals. Double breast cancers, and on one rare occasion a triple cancer, were occasionally observed in the untreated females, in about 10 per cent of the cases, whereas among the 19 test animals 5 were observed with 2, 8 with 3, 1 with 5, 2 with 6, and 1 with 7 breast cancers.

While an undoubtedly great acceleration of the development of mammary carcinoma was observed

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TABLE I: CHANGES IN 19 FEMALE MICE, DLB, PAINTED WITH METHYLCHOLANTHRENE, 0.5 PER CENT IN BENZENE, AT VARIOUS PARTS OF THE SKIN.

Mouse no.	Age at death, in months	Findings
440	8	6 mammary carcinomas, in right axilla, right side of the neck, both inguinal regions, all about 15x15x18 mm., also 2 small ones in perineum. No metastases.
442	10	3 mammary carcinomas, towards the back of the neck, 26x24x20 mm., in both axillae 10x10x10 mm. No metastases. Adenocarcinomas.
443	8	5 mammary carcinomas, in left axilla 27x 24x18 mm., ulcerated, right axilla, 18x 15x10 mm., perineum 10x8 and 10x6x 3 mm. Spleen slightly enlarged normal. Heart large, pale, myocarditis with small abscesses. Metastasis in the lung. Adenocarcinomas.
445	8	3 mammary carcinomas, in left axilla 5x 5x5 mm., left inguinal region 14x3x 10 mm., and right inguinal region, 30x20x18 mm., ulcerated. Adenocarcinoma with metaplasia to squamous cell epithelium. No metastases.
446	5½	Mammary carcinoma, in perineum 20x11x 11 mm. No metastases. Adenocarcinoma.
450	10½	3 mammary carcinomas, in right inguinal region, right axilla, 7x5x4 mm., left axilla, 6x5x5 mm. No metastases. An adenocarcinoma of the usual type, 2 mammary carcinomas with great metaplasia to squamous cell epithelium, partly with cornification.
451	7	2 mammary carcinomas, in right and left axillae, 15x15x10 and 18x11x10 mm. No metastases. Adenocarcinoma.
454	10	3 mammary carcinomas, in left axilla, 22x 14x11 mm., left inguinal region, 16x 14x12 mm., right axilla 10x10x10 mm. Adenocarcinomas. One of these with metaplasia to squamous cell epithelium. The mouse previously had small nodes, presumably papillomas, on the snout and cheek. These disappeared spontaneously. No metastases.
455	10½	2 mammary carcinomas, in the left side of the neck, 17x17x10 mm., right inguinal region, 23x22x17 mm. No metastases. Adenocarcinomas.
457	8	2 mammary carcinomas, in right side of the neck, 12x12x9 mm., left inguinal region 19x14x10 mm. No metastases. An adenocarcinoma with patchy metaplasia and a solid squamous epithelial carcinoma, apparently originating from mammary glands.
458	6	Mammary carcinoma. Leukemia. An adenocarcinoma, 21x18x18 mm. in right inguinal region. No metastases. Spleen large, 24x7 mm., dark. Liver slightly enlarged. Lymphnodes normal. Lymphogenous leukemic infiltration in liver and spleen.
459	10	2 mammary carcinomas, on the right side of the neck, 19x13x12 mm. and in perineum, 12x10x8, and right axilla, 27x21x14 mm. No metastases.
469	7	7 mammary carcinomas from 8 to 17 mm. in diameter along the lymphatics on the left side of the neck, in left axilla, left inguinal region, 2 in perineum, right side of the thorax, right inguinal region.
472	8	3 mammary carcinomas, in perineum, 28x 16x14 mm., right inguinal region, 15x 13x6 mm., right axilla, 6x6x6 mm. No metastases. Adenocarcinomas.
473	10	2 mammary carcinomas, in right axilla, 29x21x15 mm., and left axilla 5x5x 4 mm., both ulcerated. No metastases. The large tumor an adenocarcinoma of the usual type, the small one a solid mammary carcinoma without metaplasia to squamous cell epithelium.
474	7	2 mammary carcinomas, in right axilla, 26x22x15 mm., and right inguinal region, 8x7x7 mm. No metastases. Adenocarcinomas with diffuse transition to squamous epithelial carcinoma.
478	9	2 mammary carcinomas, in right axilla, 27x18x8 mm., right inguinal region, 12x10x10 mm., and in left axilla, 14x 14x10 mm. Spleen and liver slightly enlarged, thymus normal, lymphnodes about 6 mm., large. No metastases. Adenocarcinomas, one with a sarcoma-like stroma very rich in cells. Leukemoid reaction in liver, spleen, lymphnodes.
481	8½	6 mammary carcinomas, in left side of neck 20x15x15 mm., ulcerated, in both axillae, both inguinal regions, and in perineum, tumors 10 to 18 mm. in size. No metastases. Adenocarcinomas with varying, very pronounced metaplasia to squamous cell epithelium.
493	8	3 mammary carcinomas, in both inguinal regions, 45x22x22 mm. and 14x12x 8 mm. Some metastases in the lungs. Adenocarcinomas with metaplasia to squamous cell epithelium. Myocarditis with diffuse abscesses.

in the females, as was evident not only in the increased number of tumors in the individual animals, but also in the generally increased incidence and earlier appearance of the tumors, the same treatment applied to 26 males of the same strain did not produce any case of breast cancer.

Two of the 26 males died from intercurrent diseases; the remaining 24 animals had all survived 9 months of experiment when they were 10 months old, whereas nearly all the females had by then died from mammary carcinoma. Later, at the age of 11 or 12 months, 6 of these male animals had died with small or large skin papillomas situated on the snout, cheek, neck, breast,

or abdomen. Large ulcerated skin carcinomas with much cornification were found in a couple of them; these must, presumably, be considered as local painting tumors. In no case was breast cancer or leukemia observed.

TABLE II: DISTRIBUTION OF AGES AT DEATH OF 19 DLB MICE TREATED WITH METHYLCHOLANTHRENE AS COMPARED WITH 19 UNTREATED MICE DYING OF SPONTANEOUS MAMMARY CARCINOMA

Age at death in months	6	7	8	9	10	11	12	13	14	15	16	17	Total number of mice
Mice treated with methylcholanthrene	2	3	6	2	4	2	..	..	..	..	..	..	19
Untreated mice with spontaneous mammary carcinoma	..	..	..	1	1	2	3	3	4	4	..	1	19

DISCUSSION

The development of mammary carcinoma in inbred strains of mice is dependent on at least 3 factors: hereditary disposition; hormone milieu, especially estrogenic hormones; and a factor, as yet unexplained, that is transmitted through the mother's milk during the suckling period. Observations on these subjects and reviews of the literature have been presented in the papers of Bittner (1), Lacassagne (10), and Gardner (8).

In addition to these factors, it is necessary to take into consideration exogenous influences in many cases. Carcinogenic hydrocarbons are the best known examples of these exogenous influences but there undoubtedly are others. While many investigations have been made into the importance of heredity and the influence of mother's milk, and the significance of estrogenic hormones in the development of breast cancer has been much discussed, exogenous influences have rarely been studied with reference to the development of breast cancer.

It is certainly doubtful whether any of these factors that have been shown to be of significance in the development of mammary carcinoma can, *per se,* produce cancer. The malignant tumor does not develop until 2, or all 3, of the factors act simultaneously. It is possible, furthermore, that these 3 influences, even in cooperation, cannot produce a tumor without some exogenous influence, which, however, need only be very slight. From the experiments reported here, there can be no doubt that exogenous carcinogenic influences may be of importance, even of great importance, in the development of mammary carcinoma in mice.

Previous research has rarely shown such acceleration of the development of tumors. Kreyberg (9) and Bonser and Connal (3) painted mice of Kreyberg's white label strain with tar, but found no influence on the mammary carcinomas occurring spontaneously in this strain. Cramer (5) found in similar experiments a rather lower incidence of mammary carcinoma in tarred mice than in untreated animals. Dobrovolskaïa-Zavadskaïa and Adamowa (6) also found that the incidence of breast cancer was unchanged, or even lower, when a strain with about 50 per cent mammary carcinoma was treated with tar, 1,2,5,6-dibenzanthracene, or radon.

On the other hand, Maisin and Coolen (12) reported that painting with methylcholanthrene and with benzpyrene, in addition to producing tumors at the site of painting, accelerated the development of mammary carcinoma to some extent; but it is difficult to judge the results of their experiments, as there is no mention of controls.

Experiments conducted in 1939 at the Marie Curie Hospital (11) showed that estrone alone was not able to produce mammary carcinoma, but the simultaneous action of estrone and 1,2,5,6-dibenzanthracene produced a number of breast cancers, the carcinogenic hydrocarbons reinforcing, or supplementing, the action of estrone.

Such considerable acceleration of the development of mammary carcinoma by the influence of carcinogenic hydrocarbons as in the experiments here described has, however, not been observed before. It is difficult to explain why the previous experiments, mentioned above, did not show a similar acceleration of the development of tumorous processes. It may be that the various carcinogenic substances do not act uniformly in this respect, or that the mode of application and the dosage of the substance are important. The facts are still somewhat obscure. In previous experiments (7) 7 cases of acceleration of mammary carcinoma, 3 cases of leukemia, and several cases of papilloma or carcinoma at the site of painting were produced in 16 female mice of the same strain as those in the experiments reported here, by painting the skin of the back 2 or 3 times a week with 9,10-dimethyl-1,2-benzanthracene. In the present experiments mammary carcinoma appeared early, and was often multiple, in all the females painted on various sites with methylcholanthrene. The difference may perhaps be explained by the method of painting, for it is possible that the avoidance of local tumors at the site of application may explain the increased number of mammary carcinomas. These experiments, however, shed no light upon the undetermined question of the antagonism between tumors produced by painting and mammary carcinoma.

The difference between the results obtained by Mider and Morton (13) from painting dilute brown mice with methylcholanthrene and the results reported here is still more extraordinary. Exactly the same technic and the same carcinogenic hydrocarbons were employed in both experiments. While Mider and

Morton obtained a large number of accelerated leukemias in their experiments, accelerated mammary carcinomas appeared in the present experiments in all the females, whereas only 1 case of leukemia developed. Since the same technic was used for both experiments, the difference must be sought in the strains of mice employed; both of these were certainly dilute brown strains, but they were nevertheless different, since Mider and Morton stated that in their strain "breast cancer developed spontaneously in from 80 per cent to 90 per cent of the breeding females at a mean age of 10.6 months and that a 'lymphoblastoma' occurs commonly in both sexes between 650 and 800 days." In the strain used for the present experiments only about 50 per cent mammary carcinoma is observed in the breeding females and from 1 per cent to 2 per cent leukemia in the untreated animals of both sexes.

Both these strains originate from Little's dilute brown strain, and yet they are clearly distinct, both with regard to the incidence of spontaneous mammary carcinomas and leukemias and with regard to their reaction to painting with methylcholanthrene. That such a "cleavage" can occur in an inbred strain has been shown for dilute brown mice by Burrows (4), who found 4 lines with 93 per cent mammary carcinoma and 1 with only 33 per cent in 5 lines originating from 5 pairs belonging to this strain.

These facts show that the acceleration of the spontaneous tumors of a given strain that can be obtained by treatment with carcinogenic hydrocarbons is dependent on the genetic constitution of the animals. That, with regard to the mammary carcinomas, it is also dependent on the presence of estrogenic hormones, is suggested by the fact that in these experiments mammary carcinomas appeared in all the females but in not a single male. Whether it is also dependent on a factor transmitted with mother's milk is not yet known.

The experiments reported here show that the development of mammary carcinoma in an inbred strain of mice can be accelerated to a pronounced degree and can also be accentuated by the influence of methylcholanthrene. This acceleration of the development of tumors has been observed only in females, not in males. The results, together with those of other workers, indicate that this action of carcinogenic hydrocarbons is dependent on the genetic constitution of the animals.

Summary and Conclusions

Twenty-seven females and 26 males of Little's dilute brown mice were painted once a week with 0.5 per cent methylcholanthrene in benzene on various parts of the skin. All the 19 surviving females developed mammary carcinoma. Seventeen of the animals had more than 1 breast cancer. The tumors developed far earlier than is usual in this strain, the animals dying when from 8 to 10 months old as contrasted with the untreated mice which survived 13 to 15 months. No mammary carcinoma occurred in the males, but after the age of 10 or 12 months, there were some cases of papillomas and carcinomas of the skin.

Methylcholanthrene thus accelerated the development of mammary carcinoma in the females.

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Changes in the Incidence of Mammary Carcinoma in Mice of the A Stock*

John J. Bittner, Ph.D.

(From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine)

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The inbred A stock of mice has a high incidence of spontaneous mammary carcinoma. The incidence for 348 breeding females of a selected subline (F38-F58 generations) was 96.3 per cent (7, 8), for virgin females, 4.9 per cent (4).

The 41st generation A stock female No. 35904, a member of the selected line, had a cancerous ancestry for 15 successive generations and was herself cancerous. A litter of mice born to female No. 35904 was given, a few hours after birth, to a female of the CBA or X stock, No. 33562, which died without mammary carcinoma at the age of 841 days. Included in the fostered A stock litter was female No. 38432 which survived to 18.8 months and did not have mammary carcinoma. From this female a subline of A stock mice was continued which has been called the Ax strain.

Table I: Incidence of Mammary Carcinoma in Fostered A Stock Female Mice, Their Progeny, and Controls

	No. of mice	Per cent with cancer	Average age in months: Cancer	Average age in months: Non-cancer
Fostered A females	127	7.9	11.2	17.7
Progeny of non-cancer fostered females	525	6.1	12.6	16.8
Fostered Ax line, descended from No. 38432	245 *	4.5	13.9	17.2
Virgin A females	223	4.9	18.5	19.2
Breeding A females	348	96.3	10.1	...

* 111 mice, the progeny of non-cancer fostered females, are included in the fostered Ax line descended from No. 38432.

All of the 245 breeding females of the F1-F11 generations of the Ax stock have died. Their breast tumor incidence was 4.5 per cent (cancer age 13.9 months and noncancer age 17.2 months). The mammary carcinoma incidence for other fostered A stock females, their progeny, and control groups, is listed in Table I (4).

There were 11 cancerous descendants of Ax line female No. 38432. Progeny totaling 17 mice were continued from 2 of the cancerous females and they gave a tumor incidence of 5.9 per cent. Eighty-one progeny of litter mates of these cancerous mice showed an incidence of 4.9 per cent.

A representative of the 8th generation of the Ax line, female No. 62327,[1] developed a mammary carcinoma at 8 months of age. From this female the AxT line was developed. The progeny and descendants of No. 62327 are tabulated by generations, with their breast tumor ratios in Table II.

The living mice of the 11th and 12th generations were all under 11 months of age. Animals of the 13th and following generations were under observation but were too young to develop tumors.

Table II: The Development of the AxT Line of Mice and Its Incidence of Mammary Carcinoma

	Number of mice	Number living	Number cancer	Number non-cancer	Per cent cancer of total	Per cent cancer of dead
F8 (No. 62327)	1	..	1	0	100.0	100.0
F9	3	..	3	0	100.0	100.0
F10	12	..	12	0	100.0	100.0
F11	38	6	31	1	81.6	96.9
F12	48	38	10	0	20.8	100.0
Total	102	44	57	1	55.9	98.3

Four females and 1 male of the AxT line have developed breast tumors following the injection of pellets of estrone; 1 male did not develop a tumor. Ax strain mice, 24 females and 19 males, have failed to produce mammary carcinomas following the use of pellets of estrone and estradiol benzoate (8).

Summary and Discussion

Inherited mammary carcinoma in mice has been assumed to result from the interaction of (a), an active influence present in the milk of females of cancerous strains; (b), an inherited susceptibility to mammary carcinoma; and (c), the hormonal stimulation of the mammary tissue (3).

A litter of A stock mice, having a high incidence of mammary carcinoma, representatives of the 42nd

* This investigation was aided by grants from the National Cancer Institute.

[1] Included in the tumor data of the Ax stock but not its progeny and descendants.

inbred generation and with 16 successive generations of breast cancer in their direct ancestry, was fostered a few hours after birth by a female of a stock in which the incidence of mammary carcinoma was low. From 1 of the fostered A stock females a subline was continued, called Ax stock, which gave a low incidence of mammary carcinoma in breeding females. The progeny of 2 breast cancerous females of this fostered stock likewise gave a low incidence of mammary carcinoma. These observations are characteristic of the descendants of other cancerous females with fostered noncancerous mothers (2).

A female of the 8th generation in the Ax stock developed mammary carcinoma. It had not been fostered by or associated with females of the high-incidence mammary carcinoma stock. Furthermore, no breast tumors had developed in the Ax stock ancestors of this animal (average age at death for mice of the 1st-7th generations was 17.6 months). Three progeny of this cancerous female were continued and they and their descendants, called AxT line, have given an incidence of mammary carcinoma similar to that observed in breeding females of the A stock controls, which had a high incidence of mammary carcinoma.

Results obtained by Twombly (9) and Bittner (8), following the implantation of pellets of estrogenic hormones, have demonstrated that mice of the AxT line have the active milk influence for development of mammary carcinoma.

The active milk influence may be transmitted by nursing, the feeding of milk, or by the inoculation of normal tissues or organs from cancer stock donors containing the active influence (2, 5, 7, 8). Other methods of transmission are unknown.

It has been suggested that the active milk influence upon the development of mammary carcinoma is a "virus." This, however, has not been demonstrated. If this is true, the milk of low-tumor strain females may contain an inactive influence, an inactive virus, which may change (mutate?) to an active influence at any time. Or the active influence may arise *de novo* within the individual and be transferred by nursing to the progeny.

Conclusions

Mammary carcinoma in strains of mice having a high incidence of this tumor may be reduced to a minimum by foster nursing.

A line of mice with a low incidence of mammary carcinoma, but susceptible to the development of mammary carcinoma, may, without fostering by or association with mice of a strain with high incidence, acquire an active milk influence producing mammary carcinoma. Thus the line may change, in any generation, to a strain with high incidence of mammary carcinoma.

It is possible, but has not been demonstrated, for mice of a strain with low incidence of mammary carcinoma, nonsusceptible to development of breast cancer, to acquire, without fostering, an active milk influence and still not develop mammary carcinoma.

The active milk influence may arise *de novo* or by changes in the inactive milk influence.

The most consistent explanation for these observations and the data on foster nursing is that the active milk influence is an agent or virus.

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The Variability of Incidence of Mammary Carcinoma in Inbred Strains of Mice

John J. Bittner, Ph. D.*

(From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine)

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For several years this laboratory has supplied many investigators with representatives of its inbred strains of mice for research purposes. A few reports have been received stating that the incidence of spontaneous tumors, especially mammary carcinomas, has changed in the mice sent to or raised in other laboratories. It has been reported also that tumors could be transplanted in representatives of one subline of a strain whereas mice of another subline of the same stock were resistant to the grafts.

The purpose of this paper is to review publications dealing with the incidence of breast tumors and the average time of development of such growths. For various reasons reference will be made only to inbred strains of mice having a high incidence of breast tumor which are being maintained in this laboratory, namely the dilute brown, C3H, and A stocks.

The Dilute Brown Strain

The dilute brown strain of mice (Dba or D) was started by Dr. C. C. Little in 1909 and has been continued, generally by brother-to-sister matings since that date. In 1919 Little transferred his colony to Strong who returned part of the stock to Little a year later. Strong discontinued his line of this strain after a few years. From 1921 to 1925 Little's colony of dilute brown mice was under the care of E. Jones. After 1925 W. S. Murray took over the stock. Woolley assumed the responsibility following Murray's move to Springville.

The transplantation of tumors studied by Strong (22, 23) and by Little and Strong (16) demonstrated that the individuals of the dilute brown strain had attained a high degree of homozygosity by 1921, as all mice grew progressively transplants of breast tumors (dBr A, dBr B, and dBr C) which developed in mice of that stock.

Table I: Data on Mammary Carcinoma in the Dilute Brown (Dba or D) Strain of Mice

	Breeding females			Virgin females			Ovariectomized females		
	No.	Per cent cancer	Average cancer age in months	No.	Per cent cancer	Average cancer age in months	No.	Per cent cancer	Average cancer age in months
Murray (17)	479	76.6	10.5	207	11.5	15.8	210[a]	17.1	17.5
Murray (18)	1313	53.5	10.5	...	...	...	...	...	...
Murray (19)	2251	58.6	10.6	...	...	...	...	...	...
Murray and Little (20)	...	...	...	297	50.8	16.7	...	...	...
Korteweg (15)	80	76.3	?	197	72.1	?	...	...	...
Fekete and Green (14)	49[b]	81.6	10.8	...	...	...	...	...	...
Woolley, Fekete, and Little (29)	...	...	...	102[c]	39.2	15.4	82[d]	26.8	17.4
Bittner (9)	207[e]	51.7	14.2	...	...	...	...	...	...

[a] Spayed at 28 days of age.
[b] Blockage experiment, nipples sealed.
[c] 1 ovary removed at 1 day of age.
[d] Spayed at 1 day of age.
[e] Strong-MacDowell subline.

By 1930 Bittner (3) found that only 80.4 per cent of the mice of the Murray-Little dilute brown strain were susceptible to a single inoculation of tumor dBr B. Following the quadruple inoculation of the same tumor 26 per cent of the 462 mice failed to grow all grafts (4). Mice of a subline of dilute brown mice obtained by Strong from MacDowell continuously grew tumors dBr B and dBr C following transplantation. Two hundred and twenty-four pure stock animals and 191 1st generation hybrids were tested by Bittner (7).

The incidence of spontaneous mammary tumors has been reported by numerous workers since 1928. The incidence is given as the percentage of mice developing tumors which lived to the age at which the youngest animal was observed with a tumor.

Murray (17) observed that 76.6 per cent of the

* Assisted by a grant-in-aid from the National Cancer Institute.

breeding females, 11.5 per cent of the virgin females and 17.1 per cent of the females spayed at 28 days of age developed breast tumors. The numbers and average ages are given in Table I.

Additional data were published by Murray in 1930 and 1934 (18, 19) for breeding females and the respective observations were an incidence of 53.5 per cent for 1,313 mice and 58.6 per cent for 2,251 animals.

In 1935 Murray and Little (20) found that the incidence of breast tumors in 297 virgin females was 50.8 per cent.

Korteweg (15) obtained a small colony of dilute brown mice from the Roscoe B. Jackson Laboratory about 1931 and in 1936 published that the incidence in breeding females was 76.3 per cent and in virgin females 72.1 per cent. The average ages were not given although Korteweg stated that the tumors the 16th and 17th monthly age periods. The oldest mice in Murray's strain died with tumors and 82.1 per cent of the animals living to the beginning of the 17th month developed tumors (1.2 per cent of the total survived to that age). Fekete and Green observed that 12.2 per cent of their 49 mice lived to the beginning of the 17th month of which 16.7 per cent later gave rise to tumors. Four nontumorous females or 8.2 per cent of the total lived beyond the age attained by the oldest cancerous animal.

The C3H Strain

The C3H strain of breast cancerous mice was started by Strong (25) in 1920. Eighty-three breeding females of his colony gave an incidence of 69.6 per cent at an average cancer age of 13.8 months (26) (Table II).

Table II: Data on Mammary Carcinoma in the C3H Strain of Mice

Author	Subline and generation		Breeding females			Virgin females		
			No.	Per cent cancer	Average cancer age in months	No.	Per cent cancer	Average cancer age in months
Strong (26)	...	.....	83	69.9	13.8	...	....	...
Bittner (8)	...	.....	200	78.0	11.4	...	....	...
Andervont and McEleney (1)	...	F -F5	445	79.3	14.7	...	....	...
	876	F6 -F8	78	64.1	9.6	...	....	...
	492	F6 -F8	116	73.3	14.0	...	....	...
Andervont and McEleney (2)	876	F9 -F15	539	92.6	9.3	117	95.7	11.5
	876 *	F9 -F15	51	100.0	9.5	71	100.0	11.5
Bittner	...	F23-F46	351	90.0	10.6	...	....	...
	...	F23-F46	215 †	92.1	10.0	...	....	...

* Included above.
† Selected, see Figure 1.

developed a little earlier in breeding than in virgin females.

Using dilute brown females which had the right nipples sealed, Fekete and Green (14) found that these mice, when used as breeders, developed tumors in 81.6 per cent of the total.

Breeding females of the Strong-MacDowell subline of the dilute brown strain gave an incidence of 51.7 per cent for 207 animals (9). The average tumor age was 14.2 months; the ages reported by other workers ranged from 10.5 to 10.8 months.

Woolley, Fekete, and Little (29) found that 26.8 per cent of the females completely ovariectomized within 24 hours after birth developed mammary carcinoma. The incidence for females which had only 1 ovary removed at the same age was 39.2 per cent. The average tumor ages were 17.4 and 15.4 months respectively.

The highest incidence of breast tumors observed for breeding females, determined at the age of the appearance of the earliest growth, was found by Fekete and Green to be 81.6 per cent. In Murray's report (19) this incidence was only reached for the mice during

Andervont and McEleney (1) have reported on tumors appearing in a line of C3H mice obtained from Strong in 1930 but only animals born after 1932 were included. Mice of 6 generations (445 animals, F1 to F5) gave an incidence of 79.3 per cent of tumors at an average age of 14.7 months. From that time only 2 sublines were continued; namely, lines 876 and 492. Seventy-eight breeding females of line 876 of the F6 to F8 generations had an incidence of 64.1 per cent. Mice of line 492 for the same generations gave 73.3 per cent (Table II). The respective average ages were 9.6 and 14.0 months. Omission of the mice which died before the earliest age at which cancer appeared increased the tumor ratios to 70.4 per cent and 78.0 per cent.

Additional data for line 876 of the C3H strain were published by Andervont and McEleney (2) for animals of the F9 to F15 generations inclusive. The 539 females, used as breeders, gave a breast cancer incidence of 92.6 per cent at an average age of 9.3 months. Tumors developed in 95.7 per cent of the virgin females at 11.5 months. Tabulation of Andervont's data for the breeding females of the F9 to F15

generations revealed that the average tumor ages for the F9 to F12 generations exceeded 9 months whereas for the following generations the average ages were under 9 months (Table III).

TABLE III: DATA ON MAMMARY CARCINOMA OBTAINED BY ANDERVONT AND MCELENEY (2) FOR BREEDING FEMALES OF LINE 876 OF THE C3H STOCK FOR ANIMALS OF THE F9 TO F15 GENERATIONS

Generation	No.	Per cent cancer	Average cancer age in months
F9-F15	539	92.6	9.3
F9-F12	284	90.1	9.9
F13-F15	255	95.3	8.6

Litter mate controls, 51 used as breeders and 71 kept as virgins, all developed tumors (2) (Table II). The only apparent difference was that the breeding females gave rise to tumors earlier than did the virgin females (9.5 and 11.5 months).

The C3H stock mice at this laboratory were obtained from Strong during 1931 and were descendants of his female No. 59018 (F22 generation). In the first report (8), the breast tumor incidence in 200 breeding females was 78.0 per cent at an average tumor age of 11.4 months. More recent data, selected from 1 subline, indicated that the incidence was 90.0 per cent with 10.6 months as the average tumor age (Table II).

If the data are further selected to include only the 1st generation progeny of the female which is used to continue the stock in each generation and her litter mates or sisters (Fig. 1), an incidence of 92.1 per cent for 215 breeding females and an average tumor age of 10.0 months is obtained. The mice were representatives of the F23 to F46 generations. The average tumor ages decreased decidedly between the F41 and F42 generations (11.1 and 9.5 months). The latest tumor age for animals of the F42 to F46 generations was less than the earliest tumor age for the mice of the F23 to F41 generations. The separation of the data gave average tumor ages of 8.9 and 10.9 months respectively (Section A, Table III) with a slight difference in the proportion of the animals of the 2 groups which developed breast tumors.

If the noncancerous animals dying before the average cancer ages are excluded the incidence for the entire group is 97.5 per cent (Table IV, Section B). The breeding females which did not develop tumors had 19 progeny mated of which 18 or 94.7 per cent developed tumors. The nontumorous female died at 9.7 months of age. Thirty-five successive generations of mice have developed tumors in this line of the C3H strain.

TABLE IV: PREVIOUSLY UNPUBLISHED DATA ON MAMMARY CARCINOMA IN THE C3H STOCK (TABLE II) TABULATED (A) BY GENERATIONS AND (B) BY OMITTING THE NONCANCEROUS ANIMALS DYING BEFORE THE AVERAGE TUMOR AGE

General	No.	Per cent cancer	Average cancer age in months	Average non-cancer age in months	Average cancer age in months	
					Lowest	Highest
A. F23-F41	119	89.9	10.9	9.2	9.5	13.2
F42-F46	96	94.8	8.9	7.1	7.8	9.5
Total	215	92.1	10.0	8.6	...	...
B. F23-F41	111	96.4	10.9	13.0	...	...
F42-F46	92	98.9	8.9	9.5	...	...
Total	203	97.5	10.0	12.3	...	...

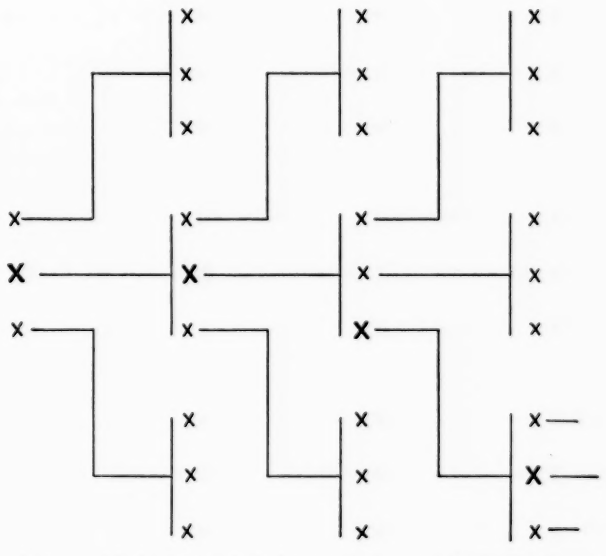

FIG. 1. Method of tabulation to observe subline differences: counting only the 1st generation progeny of the sisters of the female which is selected in each generation to continue the stock (the females chosen are designated by **X**).

THE A STRAIN

The origin of the A strain of mice has been described by Strong (27). Strong's A line descended from the 28th generation female No. 13623 (born in 1928) and was continued by its daughter No. 15317. The author's line was obtained from the same source through progeny No. 14424 and has been termed the A stock since 1931. Female No. 13623 developed a

breast tumor at the early age of 6.5 months and gave rise to the line which was selected for early cancer. The Strong A2 line, selected for late cancer, was derived from female No. 13622 (cancerous at 15.5 months). The first report by Strong (24) showed that selection was without effect. Later it was found (28) that the Strong A2 line mice developed tumors at an average age of 11.9 months whereas the Strong A line animals developed tumors at an average age of 13.8 months. Both groups received the same oatmeal diet. Differences in average tumor ages were thus noticed although selection had been made in the opposite direction. The animals which Strong maintained on a Fox Chow diet developed tumors at an average of 11.3 months.

The breast tumor incidence for 421 breeding females of the A stock was 63.9 per cent (oatmeal diet) at an average tumor age of 12.3 months (6). Following the change to the Fox Chow diet the incidence increased to 88.0 per cent (292 mice) at an average age of 11.5 months. The increase in the breast tumor incidence might be attributed to the better physical condition of the mice on the latter diet as 7.9 per cent of the mice given Fox Chow and 27.6 per cent of the animals which received the mixed oatmeal diet died without tumors before reaching the 12th month or the month nearest to the average cancer age. There is some evidence that the animals matured sooner on the Fox Chow diet as expressed by the average age of the females at the time of birth of their first litters (81 and 91 days) (10). The breast tumor incidence for 1,093 breeding females was later given as 83.6 per cent with 11.1 months being the average age (11). Only 4.9 per cent of the virgin females were cancerous (Table V).

The tabulation of a sample based on the method illustrated in Fig. 1 permits the recording of only 348 breeding females (Table VI). The average age of tumor development decreased in the 45th generation, previous to which the average age always exceeded 10 months. The incidence for the entire group was 96.3 per cent.

Thirty-eight successive generations of breast cancer have been obtained in breeding females of the A stock.

Discussion

From the data obtained by numerous investigators and from examples of changes within a single subline as cited in this paper it is apparent that the incidence of spontaneous mammary carcinoma in inbred strains of mice is variable.

Several investigations have shown that the average age at which mammary carcinoma occurs is not fixed and may change, generally to a lower average age, at any time and remain relatively constant for several or many generations. Such a variation might be considered as a "mutation." Before this interpretation is made, however, further investigations must be carried out because so little is known regarding the nature of the inherited susceptibility to breast tumors in mice. The average tumor age may be independent of the inherited susceptibility factor or factors and be governed by modifying factors.

The active influences needed for the development of spontaneous breast cancer in individual strains of mice may differ markedly. In some strains both virgin and breeding females may give rise to tumors with the only apparent difference being that the tumors develop earlier in the breeding animals. In other stocks breeding must be considered as essential for the development of tumors.

There is also some evidence that there may be at least 2 types of spontaneous mammary carcinomas in mice. The type usually characteristic of mice of inbred strains having a high incidence of mammary carcinoma has, in addition to other influences, an inherited basis. In such a stock even the progeny of females with low mammary cancer incidence may develop tumors and have an incidence approaching that characteristic of

Table V: Data on Mammary Carcinoma in the A Stock of Mice

			Breeding females			Virgin females		
Author	Subline	Diet	No.	Per cent cancer	Average cancer age in months	No.	Per cent cancer	Average cancer age in months
Strong (28)	Strong A	Oatmeal	?	?	13.8	...	...	...
	Strong A2	Oatmeal	?	?	11.9	...	...	...
	Strong A	Fox Chow	?	?	11.3	...	...	...
Bittner (6)	A	Oatmeal	421	63.9	12.3	...	...	...
	A	Fox Chow	292	88.0	11.5	...	...	...
Bittner (11)	A	Fox Chow	1093	83.6	11.1	223	4.9	18.5

Table VI: Data on Mammary Carcinoma Obtained for Breeding Females of the A Stock Selected According to Fig. 1 and Grouped by Generations with Average Tumor Ages.

Generation	No.	Per cent cancer	Average cancer age in months	Average cancer age in months	
				Lowest	Highest
F38-F34	103	95.1	12.2	10.9	13.1
F45-F58	245	96.7	9.3	7.8	9.9
Total	348	96.3	10.1	...	...

the stock. The progeny of cancerous females of low tumor strains usually show a low tumor incidence and are not cancerous like their parent. These tumors may result from changes similar to somatic mutations.

The incidence of mammary carcinomas is further dependent upon the percentage of the total number of animals living to or beyond the average breast tumor age. This is contingent upon the physical condition of the animals in their ability to attain that age; thus the physical condition may be dependent on diet. The role played by diet in bringing about maturity, as expressed by the age at which the females cast their first litters, must also be considered.

The variable tumor incidence observed by various workers for the dilute brown stock may be the expression of subline differences which have and are being recognized in this strain. These variations were first evident from studies of transplantation and are now being observed in the incidence of mammary or other types of spontaneous tumors.

In referring to a previous incidence of mammary carcinoma in a virgin female, reported by Murray and Little (20), Woolley, Fekete, and Little (29) stated:

> "This, however, probably should not be considered an absolutely accurate figure for the stock at the present time as tumor incidence different from that reported by Murray has recently been recorded in some substrains of this stock."

Future work will probably show that some lines have an incidence as high as has been reported for the other breast tumor stocks.

Inbred strains of mice are the "purest" biological material it is possible to obtain for scientific investigations. Workers not trained in genetics must recognize the fact, however, that the physiological make-up and the genetic constitution of these animals are subject to changes at any time. Animals used by one investigator should not be used as controls by another, because subline differences within a stock or variations within a single subline of a strain of inbred mice are common.

Summary and Conclusions

Observations on inbred strains of mice and on single sublines have shown that variation occurs both in the incidence of spontaneous mammary carcinoma and in the average age at which this tumor of the breast occurs. This indicates that inbred strains of mice or sublines of the same inbred stock are subject to mutational changes.

In investigations involving comparisons of inbred strains and sublines, the mice to be used as controls should be bred and reared under the same conditions as the experimental animals.

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Decline in the Incidence of Mammary Cancer in Mice of an Inbred Strain

Harold Burrows

(From the Chester Beatty Research Institute, The Royal Cancer Hospital (Free), London, England)

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The following observations were made on mice of Little's Dba strain. This strain has been maintained since 1909 by inbreeding, and after 30 years of this some constancy in the development of mammary cancer might have been expected. Yet considerable variations in the incidence of cancer have been noticed among different groups of our Dba mice, after allowing for the effects of breeding. Investigation of these discrepancies has shown that the causes may be either (a) environmental or (b) nonenvironmental. The present remarks are intended to draw attention to an instance of this latter class.

In 1935, through the kindness of Dr. C. C. Little, we received 5 pairs of Dba mice, each pair consisting of a brother and sister. From these all our stock has been derived by brother-sister matings, so that we have 5 separate lines of these mice referred to as A, B, C, D, and E. All the litters have been nursed by their own mothers. In lines A, B, C, and D, the incidence of mammary cancer has remained high. Mr. Ewers, who is in charge of the breeding room, observed at an early period that tumors did not occur so frequently among mice of the E line as among those of the other lines. Investigation proved the correctness of this observation (Table I).

Table I: Incidence of Mammary Cancer in Different Lines of Dba Mice Kept under Identical Conditions. (Only Mice Which Lived for More Than 250 Days Are Included)

Lines	Number of female mice	Number with mammary tumors	Percentage with mammary tumors	Average duration of life till tumor appeared, in days	Average duration of life in mice without mammary tumors, in days
A	19	12	63.1	418	423
B	132	76	57.5	498	487
C	134	77	57.4	441	524
D	92	52	56.5	442	478
E	69	7	10.1	439	544

The original mother of the E line mice lived 698 days and did not have a mammary tumor. There were 3 daughters and mammary cancer appeared in 2 of them. Subsequently, as shown in Table II, the tendency to mammary cancer has been small in this line. The mouse of the 11th generation which had a tumor of the breast also had a large hepatoma and a tapeworm in the liver.

Discussion

As shown in Table I, the average age at which tumors developed in the E line mice was no greater than that at which they developed in the A, B, C, and D lines. From this fact it seems that the mice of the E line have not acquired a relative insusceptibility generally distributed through the whole group; the insusceptibility appears to be either absolute or absent. No genetic investigation has been undertaken in connection with the problem. An attempt is being made to discover some difference in the organs which might be correlated with the difference of susceptibility to mammary cancer.

Table II: Incidence of Mammary Tumors in Successive Generations of Line E Mice of the Dba Strain

Generation	Number of female mice	Number with mammary tumors
1	1	0
2	3	2
3	3	0
4	5	1
5	5	1
6	7	1
7	13	0
8	13	0
9	8	0
10	8	1
11	3	1
	69	7

No cause of the divergence of the E mice from the biological behavior of the rest of the Dba stock has yet been found. The Dba mice have been kept in a room reserved for them alone. Accidental crossing with a mouse from a strain not subject to cancer can be excluded, for no aberrations of coat color have occurred, nor have we had any dilute brown mice in this institute except those of the pure strain on which these remarks are based.

From the figures which have been given, it is clear that an experiment might be seriously vitiated if mice of line E were included with the others, and the purpose of this note is to draw attention to such a possible source of error.

Summary

An unexplained decline in the susceptibility to mammary cancer in a line of inbred Dba mice is described.

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The following references to the variability of the incidence of mammary carcinoma in Dba mice have been added by the Editorial Committee:

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Studies on the Inheritance of Mammary Carcinoma in the Mouse*

Concentration of the Extrachromosomal Factor Physiological Stability of the Individual

William S. Murray, Sc. D.

(From the New York State Institute for the Study of Malignant Diseases, Buffalo, N. Y. Burton T. Simpson, M. D., Director)

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One of the more important steps in the study of the biology of cancer has been the development of a number of inbred strains of mice. The study of these stocks has shown them to be subject to the development of spontaneous mammary gland carcinoma, in greater or lesser degree. It has been found that, while there is a definite variation in the amount of cancer appearing in the different stocks, there is remarkably little fluctuation within each strain under uniform conditions of care. The constancy with which the ratios have been maintained, as generations increased, has led to the supposition that the tendency to cancer is inherited. Investigations attempting to put the occurrence of these tumors upon a definite genetic basis have led to much discussion and argument.

This controversy has been due to two basic difficulties: (*a*) the trouble experienced in establishing an inbred strain which could be proved to be free from mammary cancer; (*b*) the failure of attempts to link the disposition to cancer of any one type with the transmission of some chromosome carrying a recognizable morphological character. Because of these difficulties, the question as to the mode of transmission of the disposition to mammary cancer has never been satisfactorily settled. These investigations, however, have produced some advancement in the knowledge of this disease. Failure of recognized genetic methods to solve the problem has led to the development of other tests, which have added to knowledge and have given indication of being valuable as methods of genetic technic.

The researches of theoretical geneticists, especially those who have worked with *Drosophila* and other forms which are easily studied, lead to the conclusion that every individual is the result of the action and interaction of all of the genetic characters carried in his or her chromosomes. Thus, a certain combination of characters leads to an animal's being a mouse, while the inclusion of other characters determines whether it shall have a white coat, pink eyes, or any other character distinguishing the individual. It is not difficult to visualize a system in which many of the elements or factors are the same but which contains variables whose action on the basic factors may produce individuals showing a great variety of both morphological and physiological characters. Further, since any individual can transmit only ½ of the chromatin material which it carries, it is obvious that, with indiscriminate breeding, a great variety of genes and combination of genes may be transmitted to the young with a resultant morphological and physiological diversity. Long-continued inbreeding, on the other hand, since the young cannot be more heterozygous than their parents, and may be less heterozygous, leads to both morphological and physiological uniformity within families. Two strains developed from the same heterozygous pair may, however, be greatly different after a period of inbreeding, since the chance of the same system of genes becoming homozygous in each is remote.

Since there are at present a number of inbred strains which were not developed from the same pair of heterozygous parents and since the chance of the parents of any 2 strains being the same to begin with is very small, the probability that any 2 of the inbred families, as we now know them, have the same genetic constitution is even more remote than in the case cited above.

It follows, inasmuch as all of these inbred strains are subject to the appearance of spontaneous mammary tumors, that the tendency to have these tumors, if inherited, is due either to the interaction of those basic genes which determine the animal's being a mouse or to the presence of one or more modifying factors in reaction upon the basic mouse genes. The possibility of its being due to a single gene or the fortuitous com-

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bination of a small number of genes is extremely remote.

It does not seem unreasonable, therefore, to conclude that if the tendency to have mammary cancer is inherited, it is much more likely to be transmitted upon the basis of the interaction of the total number of genes than it is to be dependent upon a single gene or the fortuitous combination of a small number of genes. The obvious way to test this hypothesis is not by tracing the descent of individual genes or chromosomes but rather by employing tests of chromatin in the mass. A test of this type is as follows:

If females of the closely inbred dilute brown strain, which has uniformly shown a high mammary cancer incidence for a number of generations, are mated to males of the C57 black strain (the females of this latter strain have shown the greatest resistance to the development of mammary carcinoma of any stock yet developed, only a fraction of 1 per cent being cancerous), the 1st filial generation may be said to be heterozygous for high-cancer as against low-cancer. That is, they are heterozygous in those loci in which the 2 stocks differ.

On the assumption that the Mendelian theory of heredity is correct, the females obtained from the above cross should be identical in chromatin content with the young obtained by mating a C57 black female with a dilute brown male. The F1 animals in each cross receive ½ of their chromatin from the high-cancer and ½ from the low-cancer strain. According to Mendelian laws they should be identical. We find, however, that they produce spontaneous mammary tumors in significantly different numbers (1, 2). The young of the dilute brown high-cancer mothers have much more mammary cancer than do the young of the C57 black low-cancer mothers.

In the F2 generations obtained by mating the young of each of the above generations *inter se,* genetic segregation may be expected to take place and we may look for more tumors in some gene combinations than in others, if the tendency to have mammary cancer is due to the presence of a small number of genes. In the mass, however, this generation is like the previous one, in that ½ of the total chromatin is derived from the dilute brown and ½ from the C57 black stock. The findings for the F2 are the same as for the F1 generation: Those females which descended from mothers of the high-cancer stock have significantly more tumors than do those descended from the low-cancer mothers.

By the mathematical methods of genetics, it is possible to measure accurately the proportion of high-cancer and low-cancer chromatin which can be introduced into further crosses. If, for instance, females of the 2 F1 generations are backcrossed to high-cancer and low-cancer males of the parental strains, it may be said that ¾ of the chromatin in the resultant animals is derived from the high strain or the low strain, according to which male is used. If the disposition is inherited, the animals which receive ¾ of their chromatin from the high-cancer strain should have 3 times as much chance of receiving the genes necessary for the development of mammary cancer as do those which have ¼ high-cancer chromatin, and vice versa, in the reciprocal cross.

Actually, however, animals having ¾ low-cancer chromatin have just as much cancer as those which have ¾ high-cancer chromatin, if both are derived from high-cancer mothers. Those crosses which have corresponding genetic formulae but which are derived from mothers of the low-cancer strain have significantly less tumor.

Table I: Distribution of Observed Data: Development of Mammary Cancer and Deaths in Successive Months for dAF1 and AdF1 Hybrids

	dAF1 hybrids (Dbr female x albino male)				AdF1 hybrids (albino female x Dbr male)			
Month	Cancer	Dead	Total	Alive at beginning of month	Cancer	Dead	Total	Alive at beginning of month
6	1	1	2	132	..	2	2	152
7	1	1	2	130	..	1	1	150
8	..	..	..	128	1	1	2	149
9	2	1	3	128	3	3	6	147
10	13	2	15	125	1	2	3	141
11	11	1	12	110	7	1	8	138
12	15	1	16	98	8	..	8	130
13	11	1	12	82	11	4	15	122
14	13	2	15	70	9	4	13	107
15	12	..	12	55	7	2	9	94
16	7	2	9	43	14	3	17	85
17	7	2	9	34	6	4	10	68
18	10	1	11	25	21	13	34	58
19	3	2	5	14	5	7	12	24
20	4	..	4	9	6	2	8	12
21	3	..	3	5	..	3	3	4
22	..	2	2	2	..	..	..	1
23	..	..	..	...	..	1	1	1
	113	19	132		99	53	152	

It appears from these data that the tendency to have mammary tumors is transmitted by some extrachromosomal influence, which is passed on by the high-cancer mothers; that some stimulus which is not contained in the chromatin affects the cancer incidence in their young.

By making 8 consecutive backcrosses of the type mentioned above, it is possible to concentrate the chromatin of the parental generations in young derived from their hybrids, to approximate the high-cancer chromatin in animals which lack the extrachromosomal influence and to approximate the low-cancer

chromatin in animals which have this influence. If mammary cancer is inherited, those animals derived by backcrossing to high-cancer males should have as much cancer as the original high-cancer stock, since their genetic constitutions are almost identical. Animals derived in this way have, however, very little mammary cancer.

In view of these findings it is concluded that the occurrence of spontaneous mammary cancer in mice is dependent upon the presence of an extrachromosomal influence which is transmitted by the females. This being so, it is reasoned that the ratio of cancer to non-cancer in any stock may be dependent upon 2 factors: (*a*) the concentration of the extrachromosomal influence; (*b*) the reaction of mice of various physiological constitutions to this stimulus.

TABLE II: DISTRIBUTION OF OBSERVED DATA: DEVELOPMENT OF MAMMARY CANCER AND DEATHS IN SUCCESSIVE MONTHS FOR dBF1 AND ABF1 HYBRIDS

	dBF1 hybrids (Dbr female x C57 black male)				ABF1 hybrids (albino female x C57 black male)			
	Cancer	Dead	Total	Alive at beginning of month	Cancer	Dead	Total	Alive at beginning of month
5	1	8	9	116	..	..	..	...
6	..	5	5	107	..	..	..	...
7	1	3	4	102	..	..	..	...
8	..	3	3	98	..	..	..	...
9	..	1	1	95	..	..	..	...
10	1	..	1	94	1	1	2	110
11	..	1	1	93	..	..	..	108
12	1	..	1	92	2	..	2	108
13	1	2	3	91	..	1	1	106
14	5	1	6	88	2	..	2	105
15	5	1	6	82	2	..	2	103
16	4	2	6	76	1	..	1	101
17	5	3	8	70	4	1	5	100
18	..	4	4	62	4	1	5	95
19	5	5	10	58	6	1	7	90
20	..	3	3	48	7	1	8	83
21	3	1	4	45	12	4	16	75
22	1	4	5	41	4	5	9	59
23	1	2	3	36	5	4	9	50
24	2	1	3	33	4	6	10	41
25	4	4	8	30	2	6	8	31
26	1	4	5	22	2	2	4	23
27	..	4	4	17	5	2	7	19
28	..	1	1	13	4	2	6	12
29	2	3	5	12	1	2	3	6
30	..	2	2	7	1	1	2	3
31	2	2	4	5	1	..	1	1
32	..	1	1	1	..	..	..	..
	45	71	116		70	40	110	

CONCENTRATION OF THE EXTRACHROMOSOMAL INFLUENCE

From the experiments described above, it is apparent that dilute brown high-cancer mothers are capable of transmitting the extrachromosomal factor to their young and that this influence is sufficiently strong to stimulate the first generation hybrids to have tumors in appreciably large numbers. The ratio of tumor to absence of tumor in the hybrids, however, does not equal that of the straight dilute browns. The dBF1 hybrids have 60 per cent as much mammary cancer as do the inbred dilute brown females.

Since the mammary cancer ratio of the dilute brown stock stays constant from generation to generation, it appears that either (*a*) the amount or concentration of the stimulus has changed in the 1st generation hybrids, which seems unlikely, in view of its former constancy; or (*b*) the physiology of the hybrids is such that they react differently to the stimulus.

Whether or not the amount of extrachromosomal stimulus changes in the 1st generation hybrids, it is apparent that after a course of backcrossing, such as that described above, the amount or concentration does change. The 8th generation of such a series has very little of this influence, as is evidenced by the negligible tumor incidence.

That the extrachromosomal influence is variable and that different strains transmit it in different amounts is indicated by the following:

As stated above, unrelated inbred strains give various ratios of mammary cancer. If 2 of these are selected in which the percentage of tumors is large, the dilute brown and the Marsh albino strains, it is possible by the use of the method of corrected rates, to determine their relationship as regards the normal incidence of cancer. Thus the Marsh albinos have 59.9 per cent as much mammary cancer as do the dilute browns, as shown in the following tabulation:

	Dilute brown	Marsh albino
Rate	1137 per 10,000	682 per 10,000
Ratio	100	59.9

If these 2 stocks are crossed reciprocally, it might be expected that the F1 animals from both crosses would develop mammary cancer to an equal extent, since both strains have a high cancer incidence; the offspring are identical in their chromosomal content; and they all receive the extrachromosomal influence from a high-cancer mother.

By subjecting the data from these 2 crosses to the same mathematical treatment as the parent stocks, it is found that the young from the dilute brown mother and Marsh albino father cross bear the same relationship to the young from the Marsh albino mother and dilute brown father cross as the inbred dilute browns do to the Marsh albinos, as follows:

	Dbr female x albino male	Albino female x Dbr male
Rate	948 per 10,000	567 per 10,000
Ratio	100	59.8

TABLE III: CANCER RATES PER 1,000 POPULATION, PHYSIOLOGICAL DISTRIBUTION

Centiles of life span	Dbr female x albino male dA	Albino female x Dbr male Ad	Dbr female x black male dB	Albino female x black male AB
5	...	...	...	...
10	...	...	...	...
15	...	...	...	...
20	...	...	...	..
25	3	5	1	3
30	16	16	4	6
35	46	32	10	10
40	86	50	24	14
45	125	73	39	22
50	150	100	52	32
55	168	125	62	42
60	186	148	70	59
65	205	165	74	77
70	233	185	77	98
75	272	212	78	119
80	335	248	80	141
85	420	294	90	168
90	538	375	120	198
95	710	555	235	230
100	1,000	1,000	1,000	355

TABLE IV: THEORETICAL MAMMARY CANCER IN STANDARD POPULATION * PHYSIOLOGICAL DISTRIBUTION

Centiles of life span	Cross dA	Ad	dB	AB	Percentage of survivors, average virgin population
5	....	...	...	...	...
10	....	...	...	...	...
15	....	...	...	...	...
20	....	...	...	...	...
25	29	49	10	31	99.0
30	157	157	40	59	98.0
35	434	264	94	90	94.4
40	779	381	220	136	90.6
45	1,116	527	348	195	89.3
50	1,230	639	427	267	82.0
55	1,287	766	475	322	76.6
60	1,298	823	488	412	69.8
65	1,246	840	450	468	60.8
70	1,188	800	393	500	51.0
75	1,123	723	322	492	41.3
80	1,031	600	246	434	30.8
85	840	460	180	336	20.0
90	522	301	116	192	9.7
95	199	134	66	52	2.8
100	0	0	0	0	0.0
	12,479	7,464	3,875	3,986	1,316.1
Rate	948	567	294	302	...
Ratio	100	59.8	31.0	31.8	...

* The source of this standard population is to be described in another communication.

From this it appears that the amount of extrachromosomal influence is quantitative, and that some inbred strains are capable of transmitting more of it to their young than are others.

REACTION OF MICE OF VARIOUS PHYSIOLOGICAL CONSTITUTIONS TO THE EXTRACHROMOSOMAL STIMULUS

If the average length of life remaining to each animal alive at the beginning of the various chronological age periods be taken as a criterion of physiological stability for 3 unrelated inbred strains, the dilute browns, the Marsh albinos, and the C57 blacks, it is evident that the blacks are much more stable than either of the others, inasmuch as they have a life expectancy of 10.5 months when they are 14 months old, while the dilute browns and albinos have the same expectation when they are 5 and 6 months of age (Fig. 1).

Figure 2, which represents the curves for expectation of life among hybrids of these 3 stocks, indicates that the C57 blacks transmit this health and longevity to their young.

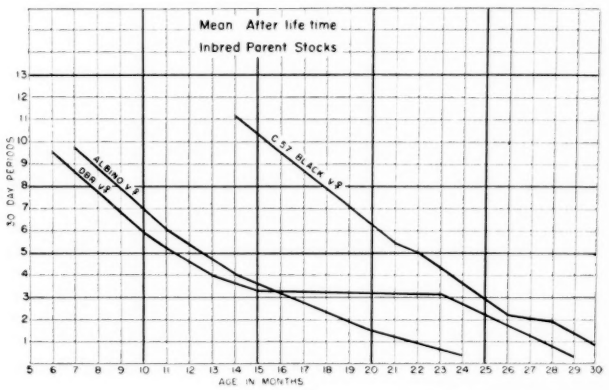

FIG. 1.—Mean life expectancy of inbred parent stocks of mice, C57 black, Marsh albino, and dilute brown.

Since it is known that the mothers influence the ratio of mammary cancer in these hybrids, an attempt should be made to measure whether or not the direction in which the cross is made has any effect upon longevity; that is, whether the maternal influence, in respect to length of life, is greater than that of the male.

The life expectancy curve for hybrids with a black parent, (Fig. 2), may be broken down according to the sex of the black parent, (Fig. 3). From this it appears that those having black mothers have a greater expectation of life than do those which have black fathers. Since, however, a large percentage of the curve for the hybrids having black fathers is influenced by the occurrence of mammary cancer in them, and that for those with black mothers is not so influenced, it is possible that the preponderance of cancer deaths in the former affects the shape of the curve and hides its true meaning. If these same curves are computed after all cancer individuals are subtracted, they arrange themselves in the positions shown in Fig. 4. From this it is apparent, since the curves cross and recross,

that it is doubtful whether or not the female influence on longevity is greater than that of the male. It is indicated, further, that the wide divergence of the curves in Fig. 3 is influenced in a measure by the cancerous females.

These graphs indicate that the physiological stability of the C57 blacks, if the ability to survive be accepted as a criterion, is determined in some measure by the chromosomes or combinations of genes carried in the chromosomes and that in the heterozygous state this

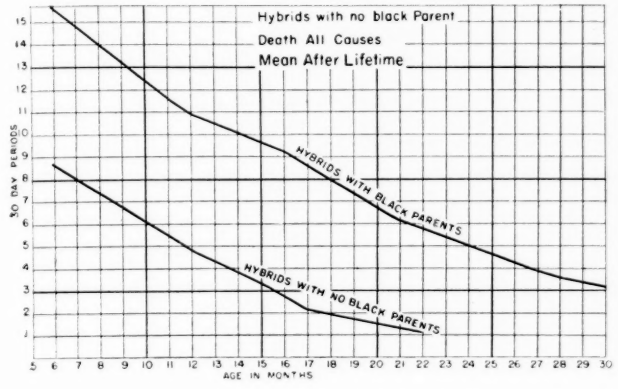

FIG. 2.—Life expectancy among hybrids of C57 black, Marsh albino, and dilute brown strains of mice. The data plotted include death from all causes.

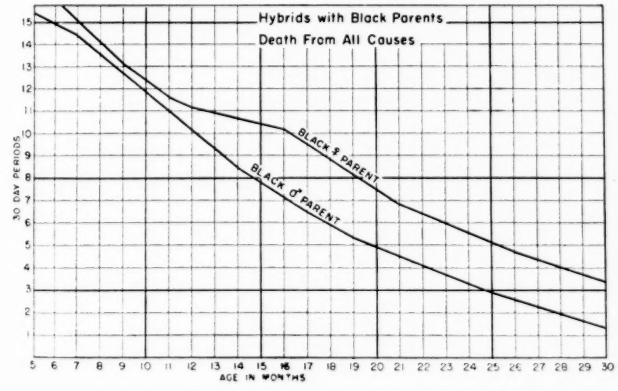

FIG. 3.—Life expectancy curves for hybrid mice with black parents.

influences the length of life of the hybrids in a plus direction. It therefore follows that hybrids having a black parent may be expected to be more stable physiologically than hybrids in which the blacks are not involved and that they may be expected to be more resistant to stimuli, external or internal, natural or artificial, than are crosses which do not carry the black chromatin.

Since the 3 parent stocks, Dbr, albino, and C57, are unrelated, they may be expected to vary in the number and position on the chromosomes of those loci in which they differ, and physiological variations may be looked for in hybrids derived from the various pairings of the parent stocks. It may be expected, for instance, that hybrids derived from albino females and black males will be physiologically different from the hybrids derived by mating dilute brown females and albino males. This may be illustrated by a graph such as Fig. 5, in which the percentage of survivors in 4 hybrid populations is plotted against chronological age. From this it is apparent that those crosses which carry black chromatin (AB and dB) have an appreciably greater life expectancy than do those crosses which do not carry the black chromatin (Ad and dA). It will also be noticed that those crosses which involve dilute brown chromatin are uniformly weaker than those in which it is not involved.

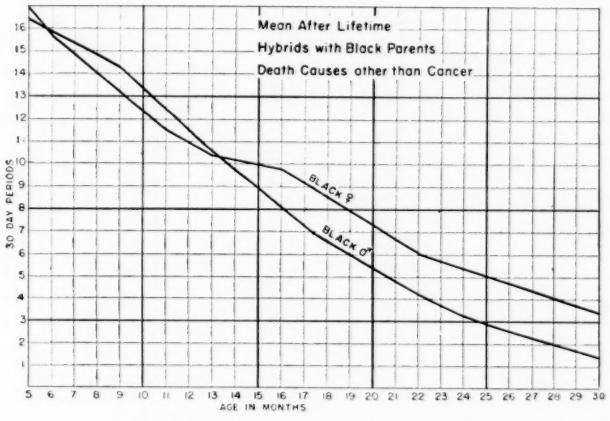

FIG. 4.—Life expectancy of hybrid mice with black parents based on deaths from causes other than cancer.

If the physiological reaction of the genetic constitution of the ABF1 generation to the extrachromosomal stimulus is the same as the reaction of the constitution of the dBF1 generation, the amounts of cancer in these hybrids should bear the same relationship to each other as does the ratio between the parent stocks (dBF1 100 to ABF1 59). When the cancer incidences for these 2 crosses are computed, it is found that they bear the ratio (dBF1 100 to ABF1 100).

This finding may be explained on either of two bases: either there is no difference in the concentration of extrachromosomal influence transmitted by the dilute brown and albino mothers, or the physiological constitution of the dBF1 generation is such that it overcomes the difference in extrachromosomal influence which was demonstrated for the parent stocks and for the Ad and dA hybrids.

If the cancer rates at the various centiles of life span of the AdF1 and dAF1 hybrids are plotted upon an arithlog grid (Fig. 6) it is found that the 2 curves are of essentially the same shape, and that the curve for the dAF1 generation is uniformly above that of the AdF1 generation. This is interpreted as indicating that the stimulus of the dilute brown extrachromosomal

influence is uniformly greater than that of the albino, throughout the corresponding physiologic ages of these 2 generations.

If similar curves are plotted for the mammary cancer rates of the ABF1 and dBF1 generations (Fig. 7) it is found that they differ from those mentioned previously in that the types of the curves are much more dissimilar; that while they give approximately equal results when the stocks are compared as a whole, these totals are reached in quite different ways. Dur-

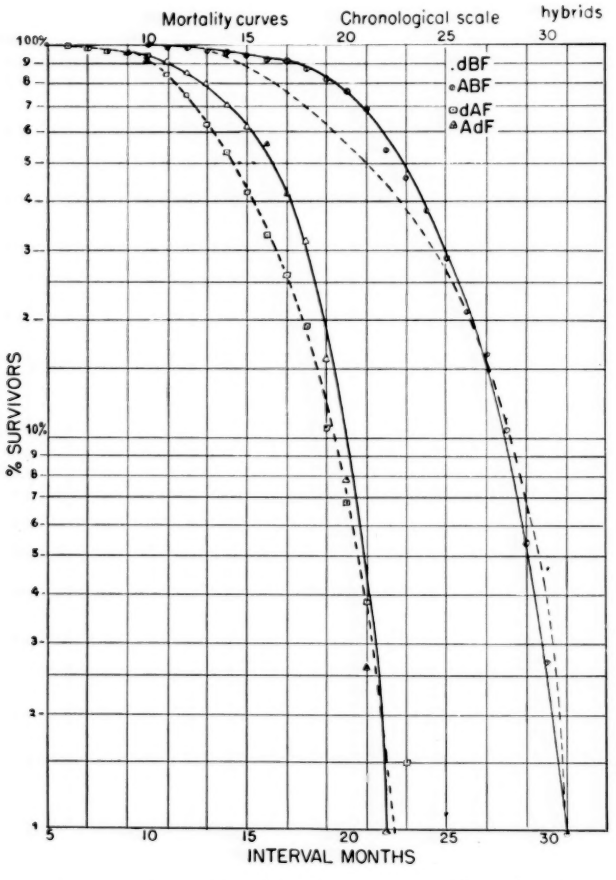

Fig. 5.—Curves showing the percentage of survivors in four populations of hybrid mice plotted against chronological age.

ing the first 65 centiles of physiologic age the dBF1 chromosomal constitution reacts with the dilute brown extrachromosomal stimulus much more readily than the chromosomal constitution of the ABF1 hybrids does with the albino extrachromosomal stimulus. Following the 65th centile these reactions are reversed.

Summary and Conclusions

1. An influence which is transmitted by the mother but not carried in the chromosomes is instrumental in causing her young to have mammary carcinoma.

2. The inbred stocks used apparently transmit this influence in degrees or concentrations which are characteristic for each stock.

3. Physiological stability, as measured by the length of life or ability to survive, is apparently transmitted through the chromatin.

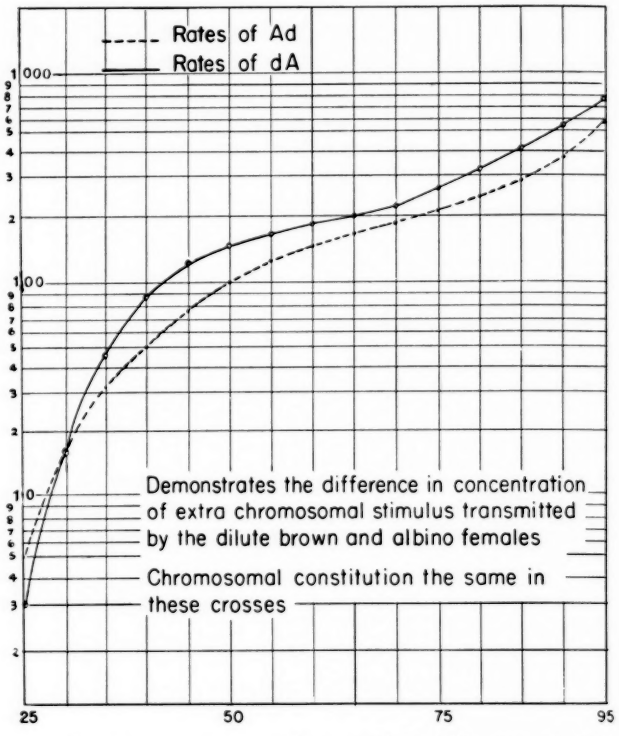
Rates of Ad
Rates of dA
Demonstrates the difference in concentration of extra chromosomal stimulus transmitted by the dilute brown and albino females
Chromosomal constitution the same in these crosses

Fig. 6.—Cancer rates at various centiles of life span of the AdF1 and dAF1 hybrid mice.

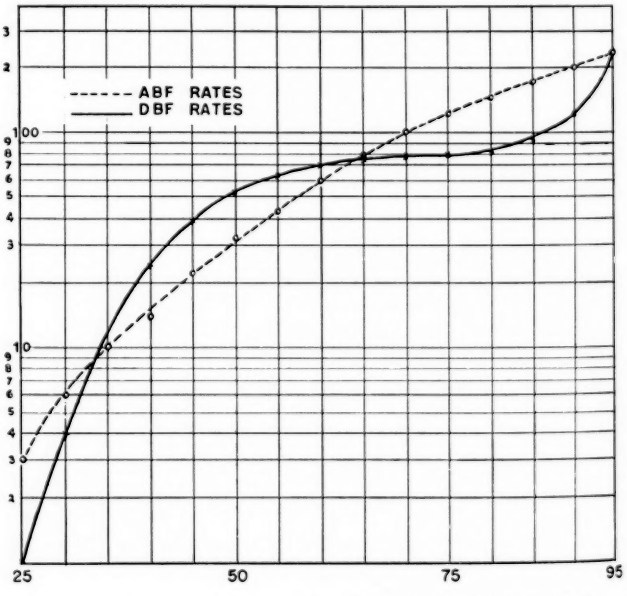

Fig. 7.—Cancer rates at various centiles of life span of the ABF1 and dBF1 generations of mice.

4. The amount of spontaneous mammary cancer in hybrids of these 3 stocks seems to be dependent upon 2 factors: (*a*) the concentration of the extrachromosomal influence transmitted by the mother; (*b*) the

physiological resistance of mice of various genetic constitutions to this stimulus.

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Environmental Temperatures and Spontaneous Tumors in Mice

R. H. Fuller, M.D., Edna Brown, and C. A. Mills, M.D.

(From the Laboratory for Experimental Medicine and Department of Pathology, College of Medicine, University of Cincinnati, Cincinnati, Ohio)

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In the course of studies upon the relationship of climate to the frequency and severity of disease we have found cancer death rates to be highest in those stimulating climatic regions where metabolic and degenerative diseases are most prevalent. On each continent for which statistics are available, cancer mortality rates closely parallel those for diabetes mellitus, being highest in middle temperate latitudes and declining sharply toward the tropics. Figure 1, prepared a decade ago but never published because confirmatory studies on experimental animals were lacking, shows the relative importance of cancer as a cause of death over the continent of North America. This map is almost identical with one similarly prepared for diabetes (3).

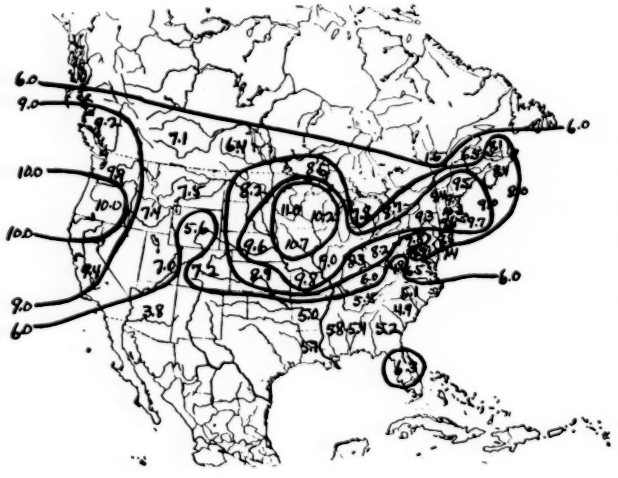

FIG. 1.—Cancer deaths as percentage of total deaths (white race only) for United States, 1924-28, and Canada, 1923-27.

Mountin and Dorn (4), in a recent study of cancer death statistics, found distinctly higher rates, both crude and age-adjusted, in New England, the Great Lakes region, and on the Pacific coast, than in the South (except for Florida and Louisiana). Gover (2) reported similar findings. According to her calculations, the age-adjusted average annual cancer mortality rates for 1930-32 in the states north of the Potomac and Ohio rivers, and latitude 37° westward, were all above 85 per 100,000, while in the southern states, with the single exception of Louisiana, they were below 80. Calculation of the crude rates for the rural white population for 1931-35 by states shows the 15 highest in the North and the 15 lowest in the South, except for 3 plateau states, Wyoming, Utah, and Idaho, with distinctly younger populations (Table I).

Mortality statistics, however, can be no more accurate than the diagnoses of the cause of death, and the majority of cancer death certificates are made out without post-mortem examination. It thus becomes highly important that any suggestion of the effect of climate upon cancer incidence be verified by controlled animal experimentation. Banzer (1) recently reported a decreased incidence and rate of growth of tumors in an animal colony which he took on a cruise from Germany into the warmth of the Caribbean, but the number of animals was small, and the data are not convincing. Our own experiments have produced rather striking results.

During 10 years' incidental observation of several thousand white mice kept at different temperature levels for other studies, the incidence of spontaneous tumors at 65-75° F was found to be between 10 and 15 per cent, while not a single tumor occurred among animals kept in a warm, moist environment. It was therefore decided to undertake more detailed observations on a special cancer strain of mice.

Two hundred females of Little's Dba strain were obtained from the Bar Harbor Laboratory and placed in experimental rooms when about 8 weeks of age. They were given Purina dog chow and water in unlimited amount. One room, the "hot room," was kept at 90-91° F. and 60-70 per cent relative humidity, another at 65° F., and a third at ordinary laboratory temperatures, ranging from 70-75° F. in winter to 94° F. on the hottest days of summer. Equal and adequate air flow through the rooms was achieved by suction ventilating systems. Lighting and all other environmental factors, except temperature, were kept as nearly identical as possible. Cooling in the cold room and heating and humidification in the hot room were achieved by use of concealed elements, so that

no direct radiational heating or cooling had to be taken into consideration.

Mice in the hot room ate only about half as much as did those in the cold, and exhibited the characteristic growth suppression which we have reported as always accompanying a disturbance in body heat loss (3). They remained in good health, however, and the death rate was considerably below that of the control group, if we except 5 animals dying during the initial period of adjustment to the high temperature. The mice were examined for tumors once monthly for the first 14 months and weekly for the remaining 4 months. Necropsy was performed as soon as possible following death. After an 18 months' observational period, accidental failure in operation of the regulating system of the hot room resulted in the death of the mice remaining in that room. All mice still alive in the cold room and control groups were sacrificed soon afterwards and the study was terminated.

Table II and Fig. 2 show the striking inhibition of tumor development in mice kept in the hot room, as compared to those in the cold room and in the control room group. Cancer development in the control group is seen to lag slightly behind that in the cold room, but the difference is negligible as compared to the suppression in the hot room. A greater difference between the control and cold room groups was found in the rate of tumor growth up to the death point. In the cold room the interval from discovery of the tumor to death of the animal averaged 28 days; in the control room the average was 47 days, while in the hot room the only 2 tumor deaths occurred 50 and 61 days after discovery of the growth, and 1 mouse was still alive over 2 months after a tumor was observed.

With 1 exception, all tumors found by examination of the living mice were adenocarcinomas, presumably originating in the scattered breast tissue. At Bar Harbor the breast tumor incidence in virgin females of the strain used had been over 50 per cent. The only tumor not of epithelial cell origin to make its presence known before death of the host appeared in a mouse in the cold room. It was of lymphosarcomatous type,

TABLE I: RURAL WHITE CANCER DEATH RATES PER 100,000 POPULATION (1931-35)

15 highest states				15 lowest states			
Massachusetts	195	Illinois	112	Arizona	27	Idaho	48
New Hampshire	158	Indiana	109	New Mexico	31	Tennessee	50
New York	139	Connecticut	109	Arkansas	38	West Virginia	50
Maine	131	California	108	Oklahoma	39	Texas	51
Rhode Island	125	Wisconsin	106	Louisiana	44	Alabama	52
New Jersey	121	Delaware	102	South Carolina	45	Wyoming	52
Michigan	115	Pennsylvania	93	North Carolina	47	Mississippi	56
		Iowa	93	Utah	47		

TABLE II: CANCER INCIDENCE IN MICE AT DIFFERENT TEMPERATURES
(Numbers in all columns are cumulative)

	Cold room 65° F.				Control room 70-75° F.				Hot room 90-91° F. 60-70 per cent relative humidity			
Date	Tumors found	Tumor deaths	Non-tumor deaths	Tumor incidence in mice not dead from non-tumor causes	Tumors found	Tumor deaths	Non-tumor deaths	Tumor incidence in mice not dead from non-tumor causes	Tumors found	Tumor deaths	Non-tumor deaths	Tumor incidence in mice not dead from non-tumor causes
7-9-38	67 mice entered				66 mice entered				67 mice entered			
2- 7-39	1	0	0	2%	0	0	1	....	0	0	5	....
3- 7-39	2	1	0	3%	1	0	3	2%	0	0	5	....
4-26-39	2	2	2	....	2	0	4	3%	0	0	5	....
6- 6-39	3	2	2	5%	2	2	6	....	0	0	5	....
7- 5-39	4	3	3	6%	2	2	8	....	0	0	5	....
8- 1-39	5	3	3	8%	3	2	10	5%	0	0	7	....
9- 5-39	7	5	11	13%	7	2	15	14%	0	0	11	....
10- 3-39	10	5	16	20%	9	5	18	19%	1	0	13	2%
10-31-39	12	10	17	24%	9	7	23	....	2	0	21	4%
11- 9-39	12	10	18	....	12	7	23	28%	2	0	21	....
11-13-39	13	10	18	26%	12	7	24	....	2	0	21	....
11-27-39	14	10	18	29%	13	8	27	33%	2	1	22	4%
1- 2-40	14	14	24	....	13	10	36	....	4	2	24	9%
1-10-40 *	14	14	25	....	13	12	37 †	....	4	2	25	....

* Experiment terminated.
† Thirteen of these mice had died with pneumonia.

involving several internal organs. Seven more visceral tumors of this same type were found post-mortem in mice showing no evidence of neoplasm before death. In addition, a papillary cystadenocarcinoma of the lung, with no discoverable extrapulmonary primary focus, was found in 2 mice, and an ovarian tumor of undetermined type in still another. It is of added significance that, of the 11 tumors involving the visceral organs, 5 were in mice kept in the cold room, 5 in the control group, and only 1 among the animals in the hot room.

Of the 67 mice in the cold room, 25 had died from causes other than cancer by 20 months of age. Exactly the same number of noncancer deaths occurred among 67 animals in the hot room, while in the control group of 66 there were 37 deaths in this same period. This excess of nontumor deaths in the control room, amounting to 50 per cent, was attributable to the high prevalence of pneumonia under the more unstable

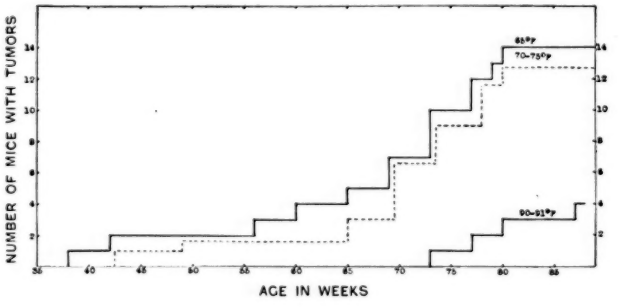

FIG. 2.—Environmental temperatures and tumor incidence in mice.

environmental conditions prevailing for these animals. Pneumonia was found at necropsy in 13 of the 37 control room mice dead of other causes than cancer but in none of the 25 animals in the hot room and in only 1 of the 25 in the cold room. This high incidence of pneumonia among mice kept under ordinary laboratory conditions, and its rarity under stabilized and controlled conditions of temperature, is a point worthy of serious consideration in experimental studies entailing many months of animal observation. It gives sharp emphasis to the value of controlled air-conditioning for animal quarters if healthy experimental material is desired.

DISCUSSION

In this study we have found a decided decrease in cancer incidence among mice kept under conditions where body heat is lost with difficulty and where tissue combustion must necessarily proceed at a slow rate. Among 67 virgin female Dba mice kept under such conditions to 20 months of age, there developed 4 breast carcinomas and a single visceral tumor classified as lymphosarcoma. Among 67 mice kept at 65° F., at which temperature body heat is easily lost, there developed 14 breast tumors and 5 tumors in internal organs. In 4 other mice in this group there were histologic changes strongly suggesting the development of leukemia. Tumor incidence in a control group of 66 Dba mice kept under ordinary laboratory conditions was almost as great as in the cold room, although the rate of tumor growth was distinctly slower. Rate of tumor growth was slowest in the mice kept in the hot room.

These findings in mice present many points of similarity to observations on human cancer. In both cases cancer incidence is significantly higher under stimulating climatic conditions that induce more rapid body growth, earlier sexual development, and more vigorous existence. It has often been suggested that the lower cancer death rate in warm countries may be due to the earlier average age of death from causes other than tumor, permitting fewer people to live on into the later decades of greater cancer incidence. This may be a factor of some importance, for infectious diseases do exact a heavier toll through the earlier decades in the tropics than in the middle temperate regions, but the cancer death rate in each of the later decades is also lower in warm regions than it is in cooler latitudes. This holds true also for the metabolic diseases, particularly diabetes mellitus (3), with which cancer has other points of similarity. A comparison of cancer death rates by age groups for 5 northern states and the white population of 5 southern states (Table III) emphasizes the difference, particularly in the later decades of life. After a maintenance of almost equal rates up to the ages of 25 to 34, there occurs an increasing divergence until the rate for the northern states becomes 45 per cent higher than that for the South (from age 65 onward). This speaks strongly against the supposition that the lower crude rates in the southern states are due merely to a delay in cancer appearance.

It is possible that the retardation of physical development due to a disturbance in loss of body heat may cause only a delay in cancer development to a later age, rather than a real decrease in total incidence. We had hoped to cover this point by observing our cancer mice throughout their natural life span, but, as stated above, our experiment was unfortunately brought to a termination when the animals had reached the age of 20 months. The character of the incidence curves (Fig. 2), however, suggests that peak development of breast tumors had already been reached in all groups before termination of the experiment. No new tumors had been found in mice in the cold room or in the

control group for over 2 months before observation ceased, and 8 of the 11 mice with visceral tumors had died before 20 months of age. The data given in Table III for human cancer deaths by age groups in northern and southern states fails to show evidence of any greater delay in cancer incidence in the South than in the North, for the rise in the death rate with advancing age is proportionately greater in the North. Instead of a delay there would seem to be a real reduction in cancer incidence in the southern states as compared to that of similar advanced age groups in the North.

The data here presented appear to afford clear evidence that ease of body heat loss and a rapid combustion rate directly affect the incidence and rate of growth of malignant tumors. Where heat loss is difficult, malignant growth is suppressed just as is body growth rate and combustion level. When body heat can be lost easily, cancer is more prevalent and its course more rapid. These findings appear to link cancer quite definitely to general tissue combustion activity, placing it among the metabolic diseases, along with diabetes, pernicious anemia, hyperthyroidism, and Addison's disease. Leukemia, be it considered inflammatory or neoplastic, also belongs in this group.

TABLE III: CANCER DEATHS PER 100,000 POPULATION

(Deaths of 1934 and 1935, population distribution from 1930 census data)

Age groups	0-4	5-9	10-14	15-19	20-24	25-29	30-34	35-44	45-54	55-64	65-74	75
Five northern states:												
Michigan, Minnesota, Wisconsin, North Dakota, Washington.....	3.8	2.3	2.2	3.9	6.0	10.1	21.4	57.9	179.8	411.4	830.6	1506.3
Five southern states:												
Alabama, Georgia, Louisiana, Mississippi, South Carolina (white only)	2.9	2.2	2.2	3.9	6.7	11.3	21.4	55.0	139.8	331.3	577.0	1035.4
Ratio of northern rate to southern rate.	...	...	...	...	...	...	1.00	1.05	1.29	1.27	1.44	1.45

SUMMARY

Virgin female Dba mice kept under conditions of moist warmth (90-91° F. and 60-70 per cent relative humidity) showed a striking decrease in spontaneous tumor incidence. Tumors that did appear under these conditions grew more slowly and took over twice as long to kill their hosts as tumors in animals kept at cooler, more stimulating temperatures (65° F.).

Tumor incidence at 70-75° F. was practically the same as at 65° F., but the masses grew more slowly.

The time interval from discovery of the tumor to the death of the animal was 28 days at 65° F., 47 days at 70-75° F., and ±60 days at 90-91° F.

That the lowered tumor incidence observed in warm environments represents a real suppression rather than merely a delay to a later age is indicated by a comparison of human cancer death rates by age groups in northern and southern states (Table III). Northern rates rise much more rapidly with advancing age than do those in the South.

From these findings it would seem that cancer should be classed as a metabolic disease, closely linked to tissue combustion level, as are diabetes, pernicious anemia, hyperthyroidism, and Addison's disease.

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Experiments with a Material from the Brown-Pearce Tumor*

Albert E. Casey, M.D.

(From the Department of Pathology and Bacteriology of the School of Medicine of Louisiana State University, New Orleans, La.)

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The purpose of this paper is to review work which was begun in 1929 and is still in progress on a material obtained from the Brown-Pearce carcinoma of the rabbit (1-20, 22). This material, when injected into rabbits, increases the size of the growth of the transplanted tumor and increases the number and size of its metastases.

By September, 1939 some 1500 rabbits and some 800 mice had been used. In each experiment particular care was taken to control such variables as health, housing, breeding, age, sex, diet, weight, season, and inoculum. Post-mortem examination was made of every animal, and in each of the rabbits some 50 areas of the body were examined. Methods have been developed for bio-assay of the material, and all results have been analyzed through the use of standard biometric procedures.

In most of the experiments slowly frozen and anaerobically preserved metastases or primary growths of the Brown-Pearce tumor were emulsified in normal saline solution. A single injection of 0.3 cc. of the emulsion, containing from 0.0001-0.1 gm. of the original tumor, was made intracutaneously or intratesticularly into rabbits 2 weeks before transplantation of the neoplasm. This treatment resulted in increased incidence, greater number, and larger volume of metastases, in an increased mortality, and in a decreased survival period.

In the course of the experiments it was shown that the material responsible for the phenomenon, sometimes called "xyz" factor, is filtrable through a Berkefeld V candle, withstands drying, is thermolabile (56° C.), can be preserved for some months, and differs from the spreading factor of Duran-Reynals. It was always present in the Brown-Pearce tumor and consistently influenced the growth and spread of this particular strain of malignant cells *in vivo*. It did not affect the growth or spread of Bashford carcinoma 63 or sarcoma 180 in the mouse. Similarly prepared and administered extracts of normal rabbit testicle and of certain mouse tumors did not affect the malignancy of the Brown-Pearce tumor. Experiments completed up to this time with an homologous material prepared in the usual manner from a transplantable adenocarcinoma of the rabbit (23, 24) have not revealed any evidence of action on the malignancy of the Brown-Pearce tumor (21).

Single and repeated injections of the Brown-Pearce tumor material did not induce visible local or general reactions in the rabbit. It did not affect the relative distribution of metastases. The effect could not be explained on the basis of an altered hemocytologic constitution shown to be important in the natural resistance of the rabbit to this tumor (8).

Injection of the material 2 weeks before, on the day of, or 2 weeks after the inoculation of the tumor resulted in an increased spread of metastases. Only the first of these injections was adopted as a part of the method of bio-assay of the material. It was shown that the material is present in fresh tumor tissue which has not been frozen. Injection of the material cannot be said to have produced any selective injury to various organs and tissues which was not produced by the injection of the tumor alone, the relative distribution of metastases being the same.

In conclusion, it should be emphasized that no other material has thus far been described which is uniformly recoverable from metastases and primary growths of a mammalian tumor, and which exerts a measurable specific and homologous stimulation of the growth and spread of the same mammalian tumor *in vivo* but has no such effect on any other tumor thus far tested. Similarly prepared and administered materials from certain other tumors of the mouse, the rabbit, and man have not produced a demonstrable augmentation of the malignancy of the Brown-Pearce tumor.

* The experiments described in this paper were begun in the laboratories of the Rockefeller Institute for Medical Research, and have been continued since 1934 largely through the aid of The International Cancer Research Foundation. The paper was read at the Third International Cancer Congress, Atlantic City, September 14, 1939.

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Observations on the Brown-Pearce Carcinoma in Roller Tube Tissue Cultures

G. O. Favorite, M. D., and F. S. Cheever, M. D.

(From the Department of Bacteriology and Immunology, Harvard University Medical School, Boston, Mass.)

(Received for publication November 16, 1940)

The present communication deals with observations made on the Brown-Pearce carcinoma in roller tube tissue cultures. This carcinoma, a highly malignant epithelial tumor discovered in the testicle of a syphilitic rabbit in 1920 (3), is readily transplantable from rabbit to rabbit by several routes, the intratesticular one being the method of choice. So far as is known to the authors, there is but one report in the literature concerning its cultivation *in vitro*.

In 1938 von Möllendorf (25) reported the successful propagation of the Brown-Pearce carcinoma over a 3 months' period employing the hanging drop and flask technic. The nutritive medium consisted of rabbit plasma and splenic extract, or in place of the latter testicle and lymph gland extracts. At first the addition of normal fibroblasts appeared necessary for the growth of tumor; in later subcultures it was possible to omit this step. After 3 months *in vitro* material injected into the testicle of a normal rabbit gave rise to the development of a typical testicular tumor which, in turn, was again propagated in tissue culture. *In vitro* 2 main types of cells were observed—round cells and spindle-shaped cells resembling fibroblasts. von Möllendorf believed that both types had a common origin and were involved in the development of the tumor; further, that round cells could become spindle-shaped cells, the former representing an exhausted stage and the latter a more active one. Morphologically she classified the tumor as a sarcoma and stressed the difficulty in distinguishing between sarcoma cells and normal connective tissue cells.

The roller tube method of tissue culture, originally suggested by Carrel in 1913 (4), first employed by Löwenstädt (17) in 1925, and subsequently modified by Gey (9), Gey and Gey (10), and Lewis (16), has been successfully employed for the culture of normal tissues (9, 17), for the propagation of viruses (7, 11), and for the cultivation of malignant growths *in vitro* (10, 16).

Materials and Methods

The fundamental principles of the roller tube method of tissue culture have been summarized by Lewis (16). Plasma is evenly distributed over the inner surface of a thin-walled test tube and in this are embedded fragments of the materials to be studied. Nutrient fluid is next added and the tightly stoppered tube is rotated in the horizontal position at 37°C. At desired intervals both fluid and gas mixtures may be changed.

Tissue.—The strain of the Brown-Pearce carcinoma, originally obtained from the Rockefeller Institute through the kindness of Dr. J. B. Murphy, had been carried for over a year in this laboratory by rabbit testicle inoculation. Vigorously growing testicular tumors or young metastases showing a minimum of necrosis were secured under aseptic precautions; any necrotic or nonmalignant tissue was removed and the remaining material minced into fragments of the desired size. The tissue was kept moist with Simms' solution (28) during the process.

Plasma.—Adult hens or cocks were bled by cardiac puncture or by venipuncture of a wing vein under sterile precautions, and by the addition of 0.01 per cent heparin, plasma was obtained.

Embryonic extract.—Three 10-day chick embryos, after removal of the eyes, were finely minced in 10 cc. of Simms' solution, incubated for 20 minutes at 37°C., rapidly frozen and thawed twice, and centrifuged for 10 minutes at 2400 R.P.M. The resulting supernatant fluid was pipetted off and stored in the refrigerator. The extracts were not kept longer than 12 days; in the majority of cases they were employed within 8 days.

Testicular extract.—The testicle of a normal rabbit was freed of tunica propria and of fat, thoroughly minced in 10 cc. of Simms' solution, incubated for 20 minutes at 37°C., rapidly frozen and thawed twice, and centrifuged at 2400 R.P.M. for 10 minutes. The supernatant was pipetted off and stored in the refrigerator.

Splenic extract.—Splenic extract was made in a similar fashion except that 20 cc. of Simms' solution were used for each spleen.

Serum.—Adult normal rabbits were bled from the heart, the blood defibrinated, and clear serum obtained by centrifugation at 2400 R.P.M. for 20 minutes. This was stored in the refrigerator.

Simms' solution.—This solution was prepared essentially according to the method outlined by Sanders (28).

All materials were prepared with aseptic precautions and bacterial sterility was checked before they were used for the actual tissue culture work.

Assembly and incubation of culture.—Clean, sterile 20 x 150 mm. Pyrex test tubes were employed. Five

to 8 drops of chicken plasma were added to each and spread evenly over the surface of the tube. Four to 8 fragments of the tissue to be cultured were placed in 1 or 2 rows along the long axis of the tube by means of a large bore Pasteur pipette. The total weight of pieces of tumor varied from 50 to 100 mgm. and they measured from 2 to 3 mm. in diameter. The tube was then gently rolled in a horizontal position for several minutes. If clotting had not taken place by this time, 1 or 2 drops of chick embryo extract were added. Then 1.5 cc. of the nutrient fluid (Simms' solution 6.5 parts, tissue extract 1 part, rabbit serum 2.5 parts) were added, and the tube rolled and shaken until the fluid had thoroughly moistened its entire surface. The tube was closed by a single-holed rubber stopper fitted with a short piece of Pyrex tubing, the external opening of which was closed in turn with a rubber vaccine cap. The tubes were placed horizontally in a rotating device similar to that described by Feller, Enders, and Weller (7), the rate of rotation adjusted to 20 revolutions per hour, and the entire apparatus placed in the incubator at 37°C.

Care of the cultures.—To secure optimal growth conditions it was found advisable to change the nutrient fluid daily since 24 hours' incubation generally resulted in a fall of pH from 7.6 to 7.2. A few of the later series were changed but once every other day. By means of a Pasteur pipette introduced through the Pyrex tubing the old fluid was removed. Then, with a long needle, 20 cc. of air drawn through sterile cotton into a 30 cc. syringe were introduced, followed by 1.5 cc. of the freshly prepared nutrient fluid. The tube was stoppered and immediately replaced in the rotator. The cultures required "patching" at some time between the 2nd and 9th days, the interval varying markedly among the individual tubes. This was accomplished by first draining the tube as completely as possible of its fluid contents, then adding 4 or 5 drops of plasma plus 2 drops of embryonic extract to insure prompt clotting. As soon as an even distribution of the fluid had been secured by a few moments of gentle rocking and turning of the tube, fresh nutrient fluid was added in the customary fashion. Colonies were transferred from one tube to another, or to new areas in the same tube.

Daily observations of the explants were made by use of the low power objective, and numerous photographs were taken at different stages. Further studies were made of fixed and stained total mounts obtained by removing intact sheets of plasma coagulum *in toto* from the wall of the tube. Parallel sections were made of tumor material from the rabbit before it was incorporated in roller tubes, the only technical difference being that the fixative used in this case was Zenker's rather than formalin. The photographs of this original tumor from the rabbit were compared with those made during the *in vitro* life of the explants and after fixation and staining.

Observations on the Tumor Explant in Vitro

The cellular reaction which must be considered in interpreting the behavior of any tumor *in vitro* has been emphasized by several workers (6, 14, 15, 20, 29). If the explant is obtained from the testicle, one must consider, in addition to the tumor cells, the structures of the seminiferous tubules (spermatogenic cells and those of Sertoli), the interstitial cells and the connective tissue framework, and finally, mononuclear cells. Since the Brown-Pearce carcinoma is characterized *in vivo* by rapid growth associated with early and extensive necrosis, one must choose a portion of the tumor which appears to be in a relatively good state of viability. An area which contains many healthy tumor cells will have a variable amount of connective tissue stroma in addition to segments of well-preserved normal tissue mentioned above. It is evident, too, that besides the mononuclear reaction which accompanies the neoplastic invasion, there is an additional and similar one which is associated with the presence of necrotic tissue. As an aid in the recognition of the several types of cells, normal testicular tissue was often placed in the same tube with the tumor but at some distance from it. The use of metastases as a source of explants simplifies the problem somewhat, but here, too, necrosis is an outstanding feature and presents the same complications.

Various combinations of tissue extracts and rabbit serum were tried, including chick embryo, rabbit embryo, rabbit testicle, rabbit spleen and, simplest of all, plain serum in Simms' solution. Twenty-five per cent serum in Simms' solution was capable of maintaining the explants, but the addition of testicular or splenic extract enhanced the growth appreciably. The former proved to be somewhat the better of the two, but, regardless of the medium used, the behavior of the explants varied quantitatively rather than qualitatively.

Within 24 hours the fresh explants invariably presented a narrow zone of mononuclear cells. Occasionally the cells were small enough to be considered lymphocytes, but as a rule monocytes and macrophages made up the predominant cell type. Within 48 hours "strands" of fibroblasts began to appear from the margin of the culture. At the same time, the monocytes became more numerous, larger, irregular in size, and slightly granular. During the next few days, the comparatively wide zone of monocytes was gradually replaced by a fibroblastic outgrowth. The fibroblasts were usually of the long, spindle-shaped type, but the flattened, irregular, stellate, and multipolar varieties were quite frequent. In approximately 3 weeks the

fibroblasts formed the basic framework of the migrating corona of new tissue, enmeshing a variety of other cells, among which mononuclears predominated. Control cultures of normal testicle presented the same picture save for the presence of fewer monocytes.

The center of the explant always remained opaque at first. Its margin blended with the fibroblastic zone and from it could be seen emerging round cells believed to be tumor cells for the most part. At times, it was difficult if not impossible to distinguish tumor cells from those of the mononuclear type, particularly in young cultures. Usually about the 10th day (the time varying considerably), the surface of the explants would slough, revealing solid sheets of tumor cells beneath.

As viewed by transmitted light through the curvature of the culture tube (magnification: approximately 130 ×), the tumor cell was round, uniform in size, and slightly refractile. It was invariably in apposition with others, and its shape might assume a slightly oval or elongated form thereby, because of mutual pressure. No distinction could be made between the nucleus and cytoplasm because of the small amount of the latter present in young cultures. The cell shape appeared to be permanent. No transitional forms between a round cell and a fibroblastic type were ever seen. At the periphery of explants consisting essentially of tumor cells, oval and slightly elongated forms were occasionally noted. Here the cells at times assumed the sheet-like formation so common in cultures of epithelial tumors. The frieze of tumor cells proceeding from within outward consisted of round, oval, and finally flattened cells at the margin. In addition, a few fibroblasts were usually observed. In comparison to the densely packed cells of the central area, those at the periphery appeared to be separated from each other. With time, this became more marked, apparently being due to an increase in the amount of cytoplasm.

In hemotoxylin-eosin preparations, the tumor cells did not differ much from those seen in the carcinoma proliferating *in vivo*. The cell was hyperchromatic; the nucleus was relatively large, vesicular, and presented a prominent nucleolus (Fig. 3). In older cultures, the cytoplasm tended to become granular (Fig. 4). The cell outlines were not always distinct, and in some areas presented a syncytial appearance. Mitotic figures were extremely rare in direct antithesis to the situation in the tumor proliferating *in vivo*.

A few cultures were treated with trypan blue by adding 1 drop of a 0.5 per cent solution of the dye in Simms' solution to 1.5 cc. of the nutrient fluid, which was renewed every other day. At the end of 72 hours, the dye was seen within the mononuclear cells and by the 5th day, bluish granules were noted in some fibroblasts when viewed through a blue filter. No tumor cells were observed to take up the dye.

Too frequent patching was quite detrimental to the tumor cells. Transplantation of explants or large fragments thereof invariably carried with it plasma clot. In many instances, the cells never left the old clot when transferred to their new environment. In such cases, the cells invariably perished. For this reason, transfer of explants was avoided when possible. The majority of our older cultures were maintained in the original tubes throughout their life span.

Frequently, after several weeks' growth, explants would be found to consist mainly of fibroblastic tissue. To circumvent this, 4 to 5 explants were placed in each culture tube. Invariably some of the explants would contain large masses of tumor cells. Again, it should be stressed that explants should contain neither too much necrotic debris nor, on the other hand, too much apparently healthy tumor, which may turn out to consist mainly of fibroblastic cells.

Isolation of the tumor cells in pure colonies could be accomplished in several ways. After a varying time, many of the extraneous cells would migrate from the explant, leaving behind a center of closely packed tumor cells. With sloughing off of dead material there would be left sheets of pure tumor cells (Fig. 2). Because of the rapid liquefaction of the clot by these cells, patching was often necessary. Unless this were done, large portions of the culture would disintegrate in the fluid medium or, as often happened, the cell masses would become attached to some other portion of the roller tube where they could be held in position by patching. Similar nests could be separated intentionally from the original explant by gentle teasing with a thin Pasteur pipette and then transplanted to another tube or to a fresh spot in the original one.

When tumor cells were isolated in pure culture they were quite inactive. The colony varied little in appearance from day to day (Fig. 1). The cells at the periphery might spread out slightly but it was difficult to say whether this was actual migration or merely passive movement due to the mechanical rotation of the culture tube. When well nourished, the cells remained clear, but when conditions were unfavorable they became small, shrunken, and pyknotic.

Recognition of the unstained tumor cell as it appeared in the orginal explants within the culture tube presented many difficulties. It soon became apparent that the cells of the advancing zone consisted of several types. von Möllendorf's observations in flask and slide cultures led her to believe that the tumor cells might assume round and fibrocytic forms. Since these were present in the authors' cultures also, it was originally assumed that both types might represent tumor cells. After several months' observations on over 100

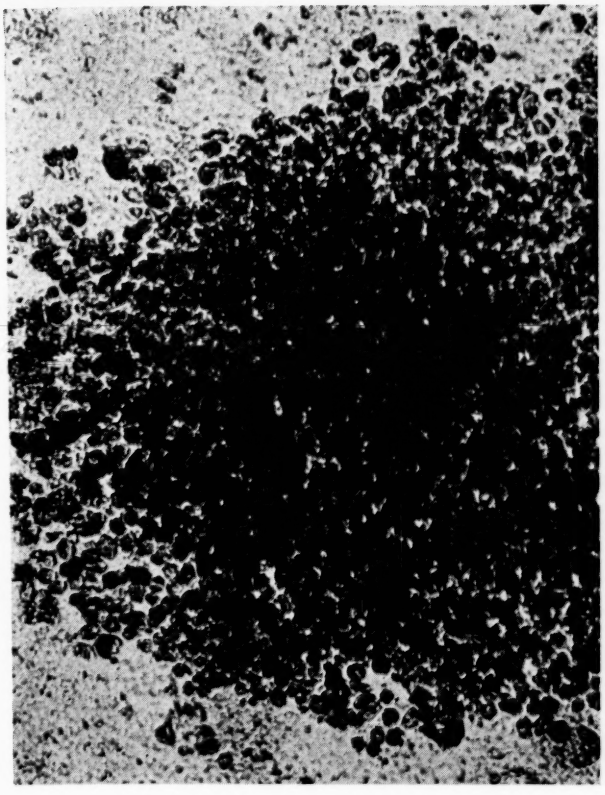


FIG. 1.—A colony composed entirely of tumor cells. Sixty-three days old. Cells show little tendency to migrate. In such colonies the cells appear merely to exist, with a certain number constantly showing degenerative changes. Unstained. × 130.

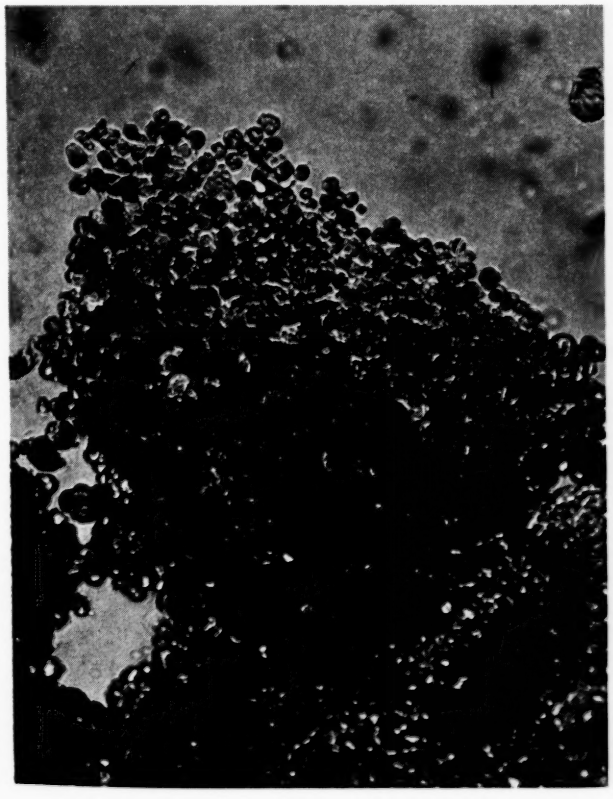


FIG. 2.—Liquefaction of the periphery of a colony, showing nests of tumor cells which can be transplanted to other portions of the same tube or to a new tube. Unstained. × 130.

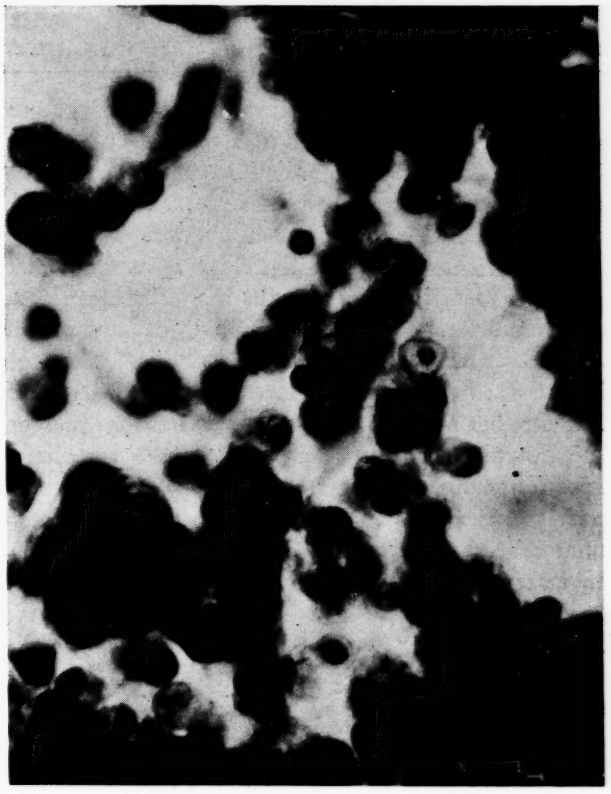


FIG. 3.—Culture, 17 days old, composed entirely of tumor cells. Nucleoli are quite prominent, while the cytoplasm is minimal. Stained. H. and E. × 600.

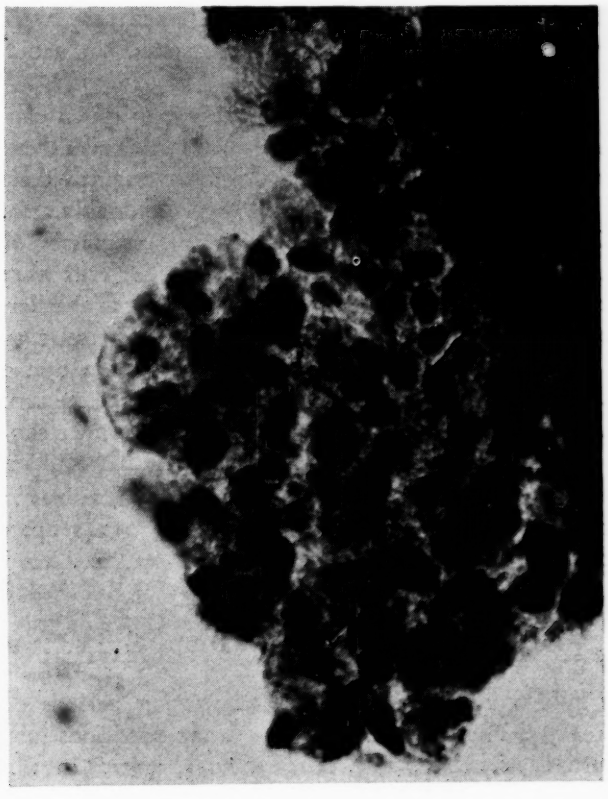


FIG. 4.—Culture of tumor, 63 days old, showing the arrangement of the cells. The granular appearance of the cytoplasm is partly due to the heavy plasma coagulum. Stained. H. and E. × 600.

cultures, it gradually became evident that a large proportion of the cells in our cultures were not neoplastic. By systematic comparison of cultures of normal testicular tissue with those of the tumor itself, it was possible to form a definite opinion on the matter.

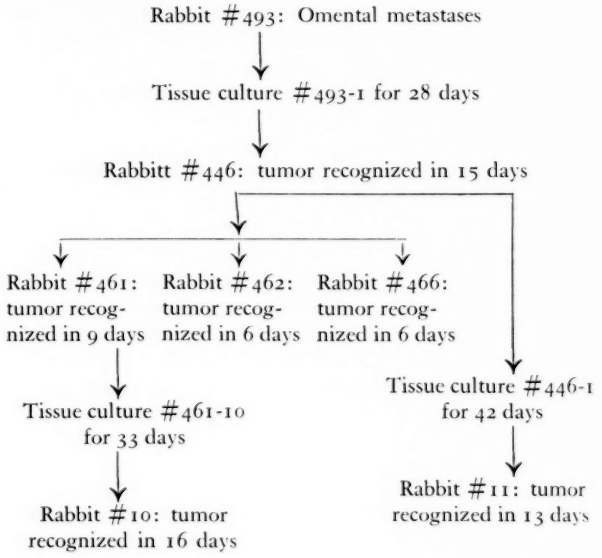
The cultures of normal testicular tissue presented an almost identical picture at the marginal zone. The predominant cell was the fibroblast, usually of the broad, flat, irregular, and multipolar variety, although the fine fusiform type was also represented. A moderate number of mononuclear cells was always present. The fibroblasts obviously arose from the connective tissue framework of the testicle, which was quite abundant. It was not possible to recognize either the interstitial or Sertoli cells, as Mendelsohn reported (24). Single seminiferous tubules were isolated, and while the cells remained in a viable state and the connective tissue wall underwent proliferative changes, the development of spermatogenic cells was not observed. In tissue sections of 10-day cultures, these cells were the first to show necrosis, while those of connective tissue origin remained in good condition. It was essential to distinguish the former structures from tumor cells, particularly since spermatogenic cells *in vivo* contained numerous mitotic figures which conceivably might develop *in vitro* also and be mistaken for tumor cells. When these spermatogenic cells were liberated in the cultures through trauma or disintegration, they showed a characteristic appearance, since they were much smaller, with considerable variation in actual size from cell to cell.

Young, small mononuclears gave rise to much confusion. At times, it was impossible to distinguish them from tumor cells. In general, the latter retained their uniformity in size and shape and were invariably associated with similar cells in the form of masses or sheets. On the other hand, the mononuclear cells were usually larger, often migrated from one spot to another, usually increasing in size and becoming irregular in outline during the process. Furthermore, the nuclei were relatively small in comparison to the cytoplasm. As the latter became granular, it made a stronger contrast to the clear nuclei.

The ability of the 2 types of cells; *i.e.*, fibroblastic and round cells, to produce malignant growths upon injection into susceptible animals will be considered in the next section.

Biological Tests of Tissue Culture Material

Tumor material cultivated for varying periods of time was injected in the form of saline emulsions into normal rabbits by the intratesticular route. In the first group, no effort was made to separate tumor cells from fibroblasts or round cells belonging to the monocyte-macrophage series. As was noted above, in spite of variations in the age of the cultures and in the type of fluid medium fed to them, in general a similar morphological picture was shown by all the cell nests. Of the 17 rabbits injected with these cultures, 10 or 59 per cent developed testicular tumors recognized clinically and proven at autopsy. A typical result is outlined in the following diagram, while the details are summarized below it.


Rabbit #493: Omental metastases
Tissue culture #493-1 for 28 days
Rabbitt #446: tumor recognized in 15 days
Rabbit #461: tumor recognized in 9 days
Rabbit #462: tumor recognized in 6 days
Rabbit #466: tumor recognized in 6 days
Tissue culture #461-10 for 33 days
Rabbit #10: tumor recognized in 16 days
Tissue culture #446-1 for 42 days
Rabbit #11: tumor recognized in 13 days

A. *Successful transfers:*

Number of culture	Age of culture in days	Number of days after inoculation when tumor was clinically recognized
492—12	17	49
444— 2	44	10
446— 1	42	13
456— 7	10	13
492— 1	17	17
492—11	17	20
492—14	17	13
493— 1	28	15
493— 2 A	14	11
461—10	33	16

B. *Unsuccessful transfers:*

Number of culture	Age of culture in days	Number of days after injection before autopsy was performed
203— 1	24	40
432— 1	63	21 *
432— 6	50	54
456— 8	10	64
456—15	10	21 *
492—16	17	52
493— 2 B	14	4 *

* Animal died of intercurrent infection.

As has been mentioned, it was possible upon occasion to isolate cell nests composed of apparently pure fibroblasts or round cells. These last were believed to be the actual tumor cells, although the possibility ex-

isted that some of them were members of the monocyte-macrophage series. Such cell nests were carefully removed and, after being ground in saline, were injected intratesticularly into normal rabbits. Positive results were obtained with 27 and 38-day old round cell cultures. Five other round cell cultures and 2 fibroblastic cultures gave negative results. The premature death of 4 animals from intercurrent infection may have reduced the number of successful inoculations. The results are summarized as follows:

Number of Culture	Type of culture	Age of culture	Result	Number of days between injection and autopsy
432—10 A	Fibroblastic	38 days	No take	63
432—10 B	Fibroblastic	38 days	No take	64
432—10 C	Round cell	38 days	Take recognizable clinically on 20th day	21
432— 1	Round cell	27 days	Take recognized clinically on 21st day	21
432— 2	Round cell	31 days	No take	14 *
432— 4	Round cell	22 days	No take	26 *
432—11	Round cell	41 days	No take	8 *
432—10 D	Round cell	38 days	No take	6 *

* Animal died of intercurrent infection.

Immune Bodies

Since Carrel and Ingebrigtsen (5) demonstrated the production of antibodies by tissues proliferating *in vitro,* later workers have reported conflicting results (26). Attempts to investigate the nature of immunity to certain transplantable tumors by this method have been complicated by the difficulty in obtaining an antiserum simultaneously effective against malignant cells and innocuous to normal cells of the host. By injecting rats with suspensions of mouse carcinoma M63, Lumsden and his associates (13, 21-23) have reported the production of a serum toxic to the malignant cells *in vitro,* while neighboring colonies of normal cells in the same tube remained unharmed. Neither Phelps (27) nor Ludford (18, 19) was able to confirm this work, and the latter has criticized it on the basis that Lumsden did not distinguish between normal and malignant cells in his tissue cultures. As it was relatively easy to procure rabbits rendered refractory to inoculations of the Brown-Pearce carcinoma, either by the intracutaneous technic first used by Brown and Pearce and later by Besredka (1, 2) or by selection of those rabbits in which testicular implantations had naturally regressed, it seemed worth while to repeat these experiments, using serums and tissue extracts from these "immune" rabbits. Furthermore, in line with Kidd's observation (12) of a complement binding substance associated with this tumor, attempts were made to demonstrate this same factor *in vitro.*

So far as the experiments went, there was no conclusive biological evidence to suggest the existence of humoral or tissue antibodies effective against tumor cells *in vitro.* Because tumor cells in pure culture lacked vigor and proliferative ability, it was necessary to use fresh explants, as suggested by Santesson (30). The effect of various combinations of serum from normal and immune rabbits plus splenic and testicular extracts from normal and immune rabbits was investigated. Three series of cultures, with proper controls, were set up and fed the immune or normal nutritive fluid for at least 10 days. No significant differences in the rate of growth were noted by direct observation and measurements, but it was diffiicult to evaluate the results, since the natural outgrowth of the explants consisted mainly of fibroblasts and mononuclear cells, which, as were expected, showed no ill effect when fed immune serum or tissue extracts. The centrally located, closely packed tumor cells did not permit accurate observations but, as far as could be seen, there was no change in their microscopic appearance to suggest that any of the media fed them had an inhibitory effect. When fragments of spleen or testicle from an immune animal were growing in the same tube with the tumor, but at some distance from it, the subsequent course of the tumor was not altered.

Nine cultures of apparently typical tumor tissue fed normal serum plus, in some cases, normal tissue extracts were injected into rabbits by the intratesticular route. Four, or 44 per cent, developed typical takes. Of 7 fragments exposed in some form to serum or tissue of immune animals, 3, or 43 per cent, gave positive results upon intratesticular injection. These 7 fragments were treated as follows:

Positive Results:

1. Fed immune serum plus Simms' solution daily for 17 days. Tumor recognizable clinically 13 days after injection.
2. Fed immune serum plus Simms' solution plus immune splenic extract daily for 17 days. Tumor recognizable clinically 13 days after injection.
3. Fed normal serum plus Simms' solution daily for 10 days. Testicular tissue from immune rabbit growing in same tube. Tumor recognizable clinically 13 days after injection.

Negative Results:

a. Fed normal serum plus Simms' solution plus immune testicular extract daily for 50 days. No take.
b. Fed immune serum plus Simms' solution daily for 10 days. Testicular tissue from immune rabbit growing in same tube. No tumor.
c. Fed immune serum plus Simms' solution plus immune testicular extract daily for 17 days. No tumor.
d. Fed immune serum plus Simms' solution daily for 17 days. Splenic tissue from an immune rabbit growing in the same tube. No tumor.

Complement Fixation

A serologically active principle associated with saline extracts of the Brown-Pearce carcinoma occurring *in vivo* has been reported by Kidd (12) to fix complement in the presence of serums obtained from rabbits bearing the specific tumor or from those in whom the growth has recently regressed. Attempts to demonstrate a similar substance from tumor grown by the roller tube tissue culture technic have met with failure. Fragments of tissue from 18 tubes (the age varying from 10 to 44 days) were carefully removed, ground up, and extracted in saline according to Kidd's technic, which was followed throughout. Six extracts proved anticomplementary and were discarded. The remaining 12 did not fix complement with any of the serums, normal or "tumor bearing," which were tried. Nutritive fluid (Simms' solution plus normal serum) in contact with the tumor cells for 48 hours gave negative results. As a last resort sterile complement and sterile serum from tumor-bearing or normal rabbits were added to the tissue culture tubes which were replaced in the incubator to be rotated for 1 or 2 hours before samples were withdrawn and added to the hemolytic system in order to test for the presence of free complement. In every case, however, complement had been destroyed apparently by the combined effect of the deteriorating temperature, 37°C, in the incubator and the anticomplementary quality of the tissue culture.

In theory it is possible that had there been more tumor material available from the roller tubes enabling one to make a more concentrated extract, fixation of complement might have been demonstrated.

Discussion

The roller tube method of tissue culture appears to furnish a technic whereby the cells of the Brown-Pearce carcinoma may be maintained for long periods of time. Direct observations carried out daily over a period of 5 months and study of the numerous photographs taken suggested that the tumor cell retained its characteristic form *in vitro*. It was felt that it could be distinguished from cells of inflammatory origin in all but young cultures. It furthermore retained its malignant characteristics, since it reproduced typical growths when injected into the testicles of normal animals after varying periods of cultivation. The longest one was 44 days *in vitro*. On several occasions it was possible to isolate cell nests consisting of pure tumor cells as far as could be determined. Upon injection of these cell masses into susceptible animals typical growths were produced in 2 out of 6 cases.

Most, if not all, of the outgrowth of tissue explants was the result of sustained proliferation of fibroblasts and mononuclear cells which made up the connective tissue stroma of the tumor. The carcinoma cells themselves remained massed in the central part of the explant, showed very little tendency to migrate, and since but 2 mitotic figures were seen in the course of 5 months' observations, one could not be certain that the malignant cells were actually reproducing themselves instead of merely persisting within the tissue explant. In connection with this, it may be remembered that von Möllendorf observed 2 types of tumor cells in her cultures of the Brown-Pearce carcinoma; *viz.,* a round cell type and a spindle-shaped fibroblastic one. On morphological and biological criteria the present authors believe that the spindle-shaped fibrocytic cells represented true fibroblasts arising from the connective tissue stroma of the tumor and that carcinoma cells *in vitro* resembled those *in vivo,* retaining their typical round cell morphology. Careful study was required in order to distinguish them from round cells of the monocyte-macrophage series.

As Fischer (8) has pointed out, some tumor cells need a framework for their growth. This appeared to be true for the Brown-Pearce carcinoma. In pure cultures, the cells showed little activity, seeming merely to exist (Fig. 1). Although they appeared to do this well, it was apparent that the mortality rate among them was high enough to insure the death of the colony in the course of time, unless multiplication occurred. Just how long these pure cultures would survive is impossible to say since at the termination of our experiments the cells were still viable as judged by their general appearance.

Immunological studies were admittedly inconclusive, but in the few experiments recorded there was no definite evidence to suggest that serum or tissue extracts from immune animals had any inhibitory effect upon the growth or malignant characteristics of the tumor cell cultivated *in vitro*.

During the course of these experiments certain shortcomings of this method of tissue culture were noted.

Rigid adherence to a routine technic and a strict observance of precautions designed to insure asepsis did not prevent a high percentage of contaminations. The use of fresh extracts and serums necessitated frequent preparations of these ingredients. This, coupled with daily changes of the fluid in the culture tubes, appeared to be the chief source of contamination.

The inability to use a higher magnification in the study of cellular details was due to the curvature and thickness of the wall of the culture tube. For the same reason, it was impossible to use dark field illumination.

All of the cultures fixed *in situ* with 10 per cent formalin were easily stripped off along with the plasma clot which covered the surface of the tube, and were for the most part stained directly after dehydration in alcohol. The young cultures were satisfactory, but the older ones became enmeshed in a relatively heavy coating of clotted plasma unless they had been transplanted several times. This coating interfered quite seriously with the study of cellular detail. A few fragments were removed *in toto* and then embedded and sectioned, but these too were generally unsatisfactory because of the thinness of the original pieces of tissue.

Summary

1. The roller tube culture technic appeared to furnish a practicable method for the maintenance *in vitro* of the specific cells of the Brown-Pearce carcinoma.

2. After 44 days in tissue culture the carcinoma cells still retained their ability to produce malignant growths in susceptible animals.

3. Definite evidence that these carcinoma cells proliferated *in vitro* was not obtained.

4. The round tumor cells retained their characteristic morphology throughout the course of the experiments and no transition to a spindle-shaped fibroblast was observed.

5. Serums and tissue extracts from animals immunized against the tumor showed no definite capacity to exert a cytotoxic action on the tumor cells *in vitro*.

We wish to express our appreciation of the aid and advice generously given by Dr. A. M. Brues of the Huntington Memorial Hospital.

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Heptyl Aldehyde-Sodium Bisulfite: Toxicity and Effect on Spontaneous Mammary Carcinoma in Mice

Frank Garai, M. D.

(From the Department of Pathology, Manhattan Eye, Ear, and Throat Hospital, New York, N. Y.)

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For the past 2 years we have observed the action of intraperitoneally or subcutaneously injected solution of heptyl aldehyde-sodium bisulfite upon mice of 3 inbred strains. All the animals had spontaneous mammary carcinoma at the time when the injections were started (Table I).

The spontaneous tumors were treated when they reached an average size of 1 cm. diameter. As tumors grow at different rates in the various strains, the tumors in the C3H mice reached an average diameter of 1 cm. in 15 days. The same size was reached in Dba mice in 18 days, and in Paris R3 mice in 10 days.

TABLE I: MICE OF C3H, DBA, AND PARIS R3 STRAINS INJECTED WITH HEPTYL ALDEHYDE-SODIUM BISULFITE

No. of mice	Strains	No. of mice injected intraperitoneally	No. of mice given alternate injections intraperitoneally and subcutaneously	Average age of mice in months	Size of tumor
25	C3H	15	10	10	0.5-1.5 cm. in diameter
25	Dilute brown: Dba	15	10	11	0.5-1.5 cm. in diameter
50	Paris R3	30	20	8	0.5-1.5 cm. in diameter
15	Control mice, no tumor	..	15	10	..

The heptyl aldehyde-sodium bisulfite was prepared by shaking a 30 per cent ether solution of heptyl aldehyde with an excess of 40 per cent sodium bisulfite solution in the cold, filtering off the crystals, and washing first with a little ice water and finally with a mixture of alcohol and ether. The product was dried in vacuum. The heptyl aldehyde-sodium bisulfite thus prepared consisted of brilliant white crystals, fairly soluble in water, and yielding a clear solution. It could be easily recrystallized from water solution but it was found that such recrystallization was not necessary.

The water solution is practically neutral and may be injected intravenously, subcutaneously, or intraperitoneally in mice without any local irritation or ulceration.

Injections were never made into the tumor or near the site of the tumor.

The toxicity of this compound was determined by intraperitoneal or subcutaneous injections in healthy mice, and intravenous injection in healthy rabbits.

Since the heptyl aldehyde-sodium bisulfite contains the equivalent of 52 per cent its weight of heptyl aldehyde all doses mentioned hereafter are calculated as heptyl aldehyde.

We found that the minimal lethal dose was 666 mgm. of heptyl aldehyde per kilo of body weight while injections of 400 mgm. per kilo produced transitory toxic symptoms. The minimum dose which produces liquefaction of spontaneous mouse tumors was found to be 166 mgm. per kilo.

This dose corresponds to 5 mgm. of heptyl aldehyde per 30 gm. mouse. The 1st injection was 3 mgm.; the 2nd, 4 mgm.; the 3rd was 5 mgm. This last dose was kept up for the remainder of the treatment. The injections were given every 2nd day regularly to 100 mice of the above-mentioned strains, each one afflicted with spontaneous mammary carcinoma. As the result of injections of the compound every 2 days for from 40 to 60 days, each animal, in accordance with its period under treatment, received from 86 to 136 mgm. of heptyl aldehyde.

A puncture section of the tumor was made on each mouse before the 1st injection of heptyl aldehyde-sodium bisulfite.

Generally, after the 1st or 2nd injection a decided expansion of the tumor occurred in all cases. After several injections the response of the tumors was not uniform. In some cases, liquefaction occurred; in other cases, regression of the tumor was noticed, and in others, hemorrhages in the tumors were observed. In those instances where liquefaction or hemorrhage occurred, fluid was aspirated from the tumor with large-gauge needles. Pathological examination of these fluids disclosed large numbers of tumor cells in each case.

In some cases where the tumors were somewhat more advanced, necrotization of the tumor tissue occurred, accompanied by a splitting of the skin and a complete dislodging of the tumor as a whole. In these instances the falling out of the tumor was occasionally followed by sufficient hemorrhage to cause the death of the animal. In other instances where no fatal hemorrhage took place suturing of the skin failed to prevent the occurrence of secondary infection, which was always fatal.

When the tumors were approximately 0.5 cm. in diameter and some hope of total regression seemed reasonable, the animals were kept under treatment for an average of 60 days, with the usual injection every 2nd day. While it was noticed that the growth of the treated tumors was retarded in comparison with the untreated control tumors of the same strain, the mice eventually succumbed. The control animals with untreated tumors died approximately 21 days earlier than the treated animals.

There were no differences in the reactions of the tumors in each strain to the heptyl aldehyde-sodium bisulfite compound.

Autopsies were made on every treated animal and pathological examination of sections from the tumor, kidneys, and liver disclosed the cause of the deaths. The tumors were more or less necrotized, but a partial or total destruction of the tissues of the kidneys and liver of all of the treated animals were the most striking features.

There were no strain differences in the resistance of the mice to the toxic action of the compound upon kidneys and liver.

Summary and Discussion of Results.

The heptyl aldehyde-sodium bisulfite aqueous solution used exerted a destructive effect on kidneys and liver. Associated with this was a partial retardation of the growth of spontaneous tumors in mice. However it is well known that any procedure which affects the general health of an animal may retard the growth of an associated tumor. The effect on the kidneys and liver may have been caused by absorption of toxic substances from the destruction of the tumor during treatment, but 15 controls with no tumor showed similar destructive effects in their kidneys and livers as a result of similar chemical treatment. Strong (1) reports no toxic results and favorable tumor reactions in mice receiving heptyl aldehyde-sodium bisulfite injections. We do not confirm these results.

Conclusion.

The destructive action of continued doses of heptyl aldehyde-sodium bisulfite upon the kidneys and liver far outweighs its effect upon the growth of spontaneous tumors, insofar as any application of this product to the chemotherapy of cancer is concerned.

Nevertheless, we feel that studies with heptyl aldehyde compounds in relation to spontaneous tumors opens up a definitely attractive field for further experimentation with compounds of this type.

We are indebted to the Department of Pathology of the Manhattan Eye, Ear, and Throat Hospital, New York City, for generous cooperation.

To Dr. A. A. Eggston, Director of this Department, we owe a special debt of gratitude for his study and description of the sections.

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Glutamic Acid in Tumor Hydrolyzates

R. G. Mathers

(From The Shrubsall Biochemical Laboratories, Westminster Hospital Medical School, University of London, London, England)

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In 1939 Kögl and Erxleben (7-9) reported that the hydrolysis of tumor tissue yields amino acids belonging partially to the dextrorotary series, whereas the naturally occurring amino acids belong to the levo series. The most striking result was obtained in the case of glutamic acid. Kögl claimed that 45 per cent of this acid is present in the d(-) form. Having regard to the central position which it is now believed glutamic acid holds in the metabolism of animal tissues this finding, if confirmed, is of unbounded importance.

This finding, with respect to glutamic acid, has been confirmed by Schroeder (10); Arnow and Opsahl (1); and White and White (11); and has been disproved by Graff (6); Chargaff (2); Dittmar (5); and Chibnall, Rees, *et al.* (3, 4).

In all cases except that of the last named workers the yield of glutamic acid has been low. In the present paper the results of the hydrolysis of 6 human tumors and 2 specimens of normal tissue is given.

An acetone powder of the tissue was made and extracted with ether. This powder was then hydrolyzed with hydrochloric acid. In the case of the bronchial carcinoma 2 methods of isolating glutamic acid were used. In the 1st the glutamic acid was precipitated as the barium salt after the di-amino acids had been removed and the hydrochloride obtained from this. In the 2nd the glutamic acid was precipitated directly as the hydrochloride from the tissue hydrolyzate. In all the other cases this 2nd method alone was used. All polarimetric readings were done in 9 per cent HCl solution.

Pure levo glutamic acid hydrochloride was submitted to the same process as the tissue and no racemization was found to occur.[1] It was, however, found necessary when concentrating the solutions of the amino acid *in vacuo* to keep the temperature of the water bath below 45° C. otherwise a loss of glutamic acid occurred. A compound soluble in glacial acetic acid was formed. The results of the analyses are presented in Table I.

TABLE I: RESULTS OF ANALYSES OF MALIGNANT AND NORMAL TISSUES FOR GLUTAMIC ACID

Tissue	Yield in mgm. per cent of tissue	Per cent of dextro-glutamic acid
Human bronchial carcinoma	63	0.89
Human bronchial carcinoma	68	0.91
Human psoas sarcoma	79	0.74
Human carcinoma stomach	71	0.70
Human carcinoma rectum	81	0.55
Human carcinoma stomach	77	0.50
Human glands from lymphatic leukemia	55	0.50
Normal ox heart	83	0.33
Normal calf liver	91	0.48

It will be seen that the amount of d(-) glutamic acid found in tumor tissue was small and only a slightly smaller amount was found in normal tissue.

I wish to thank Professor E. Friedmann of the Biochemical Laboratory, Cambridge, for his advice and encouragement during the course of this work and the John Burford Carlill trust of Westminster Hospital for paying the expenses of this work.

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The Effect of Carcinosarcoma 256 on the Water Content of the Liver

H. Douglas McEwen*, Ph.D., and Frances L. Haven, Ph.D.

(From the Department of Biochemistry and Pharmacology, School of Medicine and Dentistry, The University of Rochester, Rochester, New York)

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During the course of experiments on the growth of rat carcinosarcoma 256 when inoculated directly into the liver, the percentage of water in the liver was found to be higher than normal. This suggested the possibility that tumor grown subcutaneously might bring about the same change. Schlottmann and Rubenow (5) reported high percentages of water in the livers of rats bearing Flexner carcinoma and Jensen sarcoma. Since no reports concerning the percentage of water in the livers of rats bearing carcinosarcoma 256 were found in the literature, it seemed desirable to investigate the effect of tumor grown subcutaneously on the percentage of water in the liver.

Experimental

Young male rats of the Wistar strain, weighing from 60 to 100 gm., were inoculated with carcinosarcoma 256 subcutaneously in the groin by the trochar method. Those animals in which the tumor did not take served as controls. From 14 to 49 days after inoculation and at approximately the same time of day, the animals were sacrificed, the tumors removed and weighed and the presence or absence of metastases noted. The livers were always found free from metastases. The livers were removed, weighed, and a portion taken for water analysis; this portion in turn was weighed, macerated in a few cc. of 95 per cent alcohol and dried to constant weight in an oven at 105° C.

One group of animals which had been raised on the stock diet (G. L. F. calf meal) was maintained on this diet throughout the experimental period. Another group also raised on this stock diet was changed at the time of inoculation to synthetic diets containing various fats. The fats used were cod liver oil and coconut oil in Sinclair's Diet No. 262 (6) and elaidin in Sinclair's Diet Number 290C (7). In other experiments, cholesterol, and cholesterol and choline were added to the coconut oil diet. The cholesterol was added as 1 per cent of the diet, replacing an equivalent amount of fat; the choline was added as choline hydrochloride, the amount being approximately 90 mgm. per rat per day. The animals had access to these diets and to water at all times.

Results and Discussion

In the livers of rats on the calf meal diet the average percentage of water for the controls (73 animals) was 70.3 ± 0.8.[1] For the 31 rats bearing carcinosarcoma 256 the average percentage of water was 73.6 ± 0.9 (Fig. 1). The average for the tumor-bearing animals includes only those animals with tumors weighing over 10 gm. A similar increase was found in the percentage of water in the livers of tumor-bearing rats on all other diets, the average percentage for the 47 control animals being 69.9 ± 1.2 and for the 107 tumor-bearing animals being 73.8 ± 0.9 (Fig. 2).

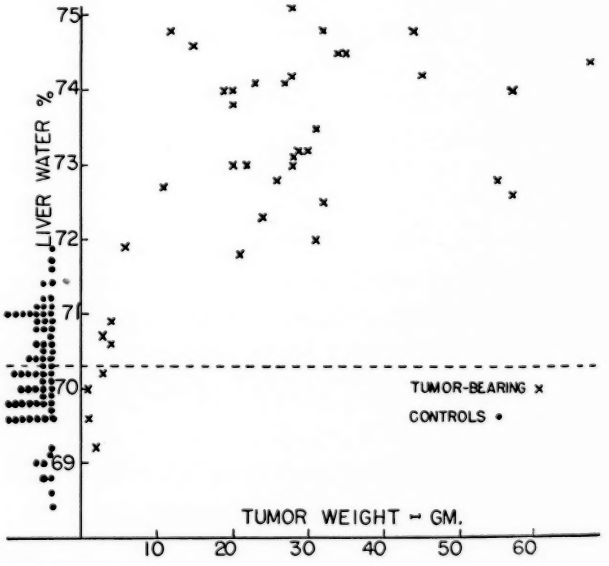

Fig. 1.—The percentage of water in the livers of control rats and of rats bearing carcinosarcoma 256 on calf meal diet. The broken line shows the average percentage of water in the livers of the control animals.

* Present address: Department of Chemistry, School of Medical Sciences, University of South Dakota, Vermillion, South Dakota.

[1] In this and subsequent averages the value following the ± sign is the standard deviation of the mean.

This difference in percentage of liver water between the control and tumor-bearing animals for both groups (Figs. 1 and 2) is statistically significant. The standard deviations were calculated using the formula

$$\text{S. D.} = \sqrt{\frac{\Sigma x^2}{n}},$$ where x = the deviation of each value from the mean. The significance was determined by dividing the difference between the means by the standard error of difference, $\sqrt{\sigma_a^2 + \sigma_b^2}$.

Fig. 1 shows that the percentage of water in the livers of rats growing carcinosarcoma 256 subcu-

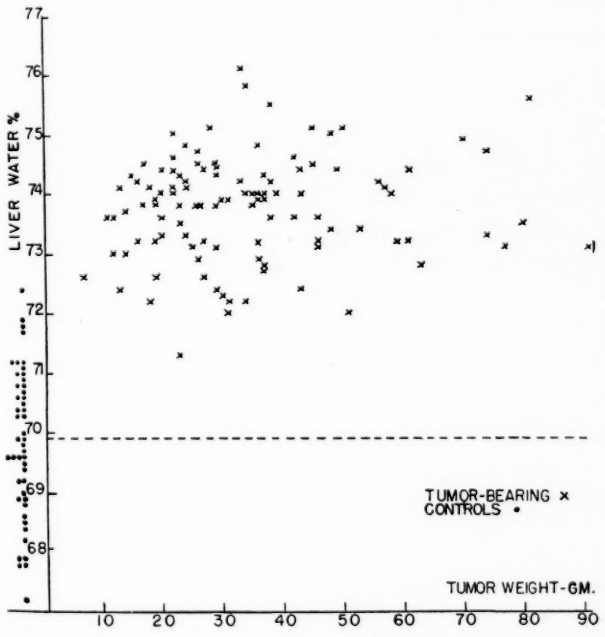

FIG. 2.—The percentage of water in the livers of control rats and of rats bearing carcinosarcoma 256 on all other diets. The broken line shows the average percentage of water in the livers of the control animals.

taneously is higher than that in the livers of rats in which this tumor did not take (controls). The average percentage of water in the livers of the control rats (70.3) is essentially that found by Fenn (2) and Bridge and Bridges (1) for normal rat livers.

This increase in water in the liver was found (Fig. 2) to be independent of the diet. It is well known that the character of the diet alters the amount of glycogen and fat deposited in the liver. According to Fenn (2) and to Puckett and Wiley (4) the amount of glycogen deposited in the rat liver may vary markedly without altering the percentage of water in the liver. On the other hand, according to Kaplan and Chaikoff (3), fat is deposited in the liver without a simultaneous deposition of water, thereby decreasing the percentage of water in the liver. It has been shown that diets containing excessive amounts of fat and cholesterol tend to produce a deposition of fat in the liver. In spite of this, the tumor-bearing rats on our fat-containing diets with and without choline showed an increased percentage of water in the liver (Fig. 2).

Our results indicate (Fig. 1) that the tumor must have attained a certain size (10 gm.) before this alteration in percentage of water in the liver occurred. After this, increase in tumor size caused no further

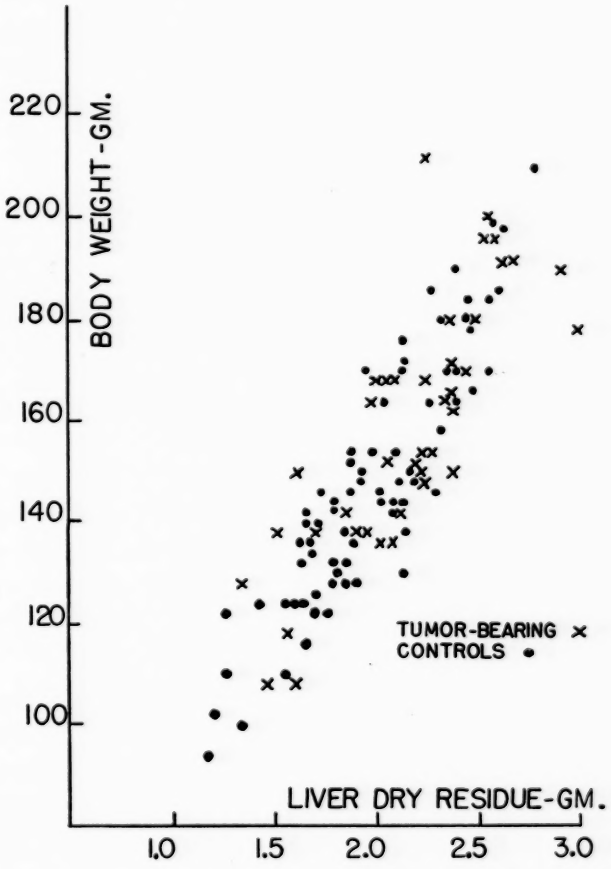

FIG. 3.—The relation between the liver dry residue and the body weight in control rats and in rats bearing carcinosarcoma 256 on calf meal diet.

increase in percentage of water in the liver. One might expect the increase in percentage of water in the liver of tumor-bearing rats (Figs. 1 and 2) to be the result of a decrease in liver dry residue. That such is not the case is shown in Fig. 3 in which the total liver dry residue is plotted against the total body weight for the tumor-bearing and control rats on calf meal diet.

Since there has been an increase in the percentage of water without a corresponding decrease in dry residue in the livers of the tumor-bearing rats, the weight of the liver per 100 gm. of body weight should be greater in these rats than in controls of comparable body weight.

Fig. 4 shows the relation between body weight and liver as per cent of body weight for all animals on the calf meal diet. Although the liver as per cent of body weight appears to be higher in the tumor-bearing animals the difference between the 2 groups is not statistically significant. It is apparent that the presence of tumor influences this relationship.

In Fig. 5 is shown the relation between the liver as per cent of body weight and the weight of the tumor. The increase in size of the liver is most apparent in animals bearing tumors weighing from 10 to 40 gm. As shown in Fig. 1, increase in the percentage of water does not occur until the tumor weighs over 10 gm., so less change in the relation of liver to body weight might be expected in this group. At present we have no adequate explanation for the normal figures for liver as per cent of body weight in the animals bearing tumors weighing over 40 gm. Similar relationships hold for the tumor-bearing animals on all other diets.

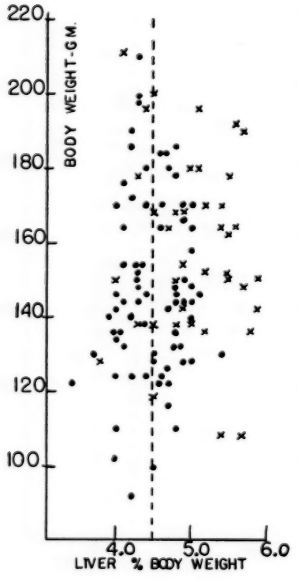

Fig. 4.

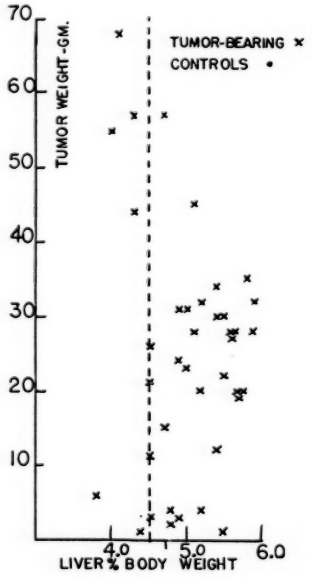

Fig. 5.

Fig. 4.—The relation between the size of the liver and the body weight in control rats and in rats bearing carcinosarcoma 256 on calf meal diet. The broken line shows the average size of the liver as per cent of body weight for the control rats.

Fig. 5.—The relation between liver size and tumor size of rats bearing carcinosarcoma 256 on calf meal diet. The broken line shows the average size of the liver as per cent body weight for control rats.

Consideration was given to calculations of the data shown in Figs. 4 and 5 in terms of total body weight minus weight of the tumor. In view of the effect of the tumor on the whole organism it was decided to regard the tumor as a part of the animal rather than as something extraneous to it. However, when the data shown in Fig. 4 are recalculated on the basis of body weight minus weight of the tumor, the differences between the control and tumor-bearing animals are not statistically significant. The data in Fig. 5 recalculated on the basis of liver as per cent of residual body weight show that the values for the animals bearing tumors over 40 gm. no longer fall along the average for the controls (4.5 per cent) but do not exhibit the trend of increase in liver as per cent of residual body weight with increase in tumor weight as shown by the other tumor-bearing animals. This, however, still fails to explain why the figures for animals bearing these large tumors do not more closely resemble those for the other animals.

Summary

The percentage of water in the livers of rats bearing carcinosarcoma 256 was found to be significantly higher than that in the livers of normal rats. This increase in liver water occurred independently of the diets used and is not accounted for by a decrease in dry residue.

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The Transformation of Rat Mammary Adenofibroma to Fibroma by Androgens*

Frederic E. Mohs, M.D.

(From the McArdle Memorial Laboratory, Medical School, University of Wisconsin, Madison, Wisconsin)

(Received for publication November 16, 1940)

In the course of experiments on the hormonal factors influencing the growth and transplantability of mammary adenofibroma in rats (6) it was observed that in males the tumors often become pure fibromas, while in females the tumors retained their original adenofibromatous structure. The objects of the present experiments were, first, to determine the hormonal factors responsible for the fibromatous transformation; second, to determine the time required for the transformation to occur; and third, to determine the constancy of the phenomenon in different strains of transplantable adenofibroma.

Since these experiments were started other workers have reported changes in the morphology of mammary adenofibroma under various conditions. Thus, Heiman (3) reported a relative increase of fibrous tissue in adenofibromas growing in old males, in castrate females with or without gonadotropic hormone injections, and in castrate males receiving either gonadotropic or estrogenic hormone injections. Heiman (4) also reported inhibition of the glandular components of adenofibroma by large doses of androgens. Murphy *et al.* (8) found that estrogens failed to prevent the fibromatous transformation of adenofibroma growing in males.

Experimental

Grafts of actively growing tumor tissue 8-10 mm. in diameter, obtained from 11 different strains of transplantable mammary adenofibroma were implanted in the left groin of the following groups of rats: (a) normal young mature males and females, (b) castrates of both sexes, and (c) castrates of both sexes receiving estrogens and androgens. These rats were obtained from the local "Sprague-Dawley" colony, and are not closely inbred, though no outside strains have been introduced for over 14 years. The 11 strains of transplantable adenofibroma originated as spontaneous tumors in rats of the same colony, the 1st transplant generation being used in 8 of the strains, the 1st and 2nd generations in 2 and the 1st to 6th generations in 1.

Estrogen-injected groups received 6 rat units of Amniotin [1] in oil every other day starting 2 days before implantation and continuing for the duration of the experiment. Androgen-injected groups received 0.25 mgm. of testosterone propionate [2] in oil twice a week starting 2 days before tumor implantation and continuing for the duration of the experiment.

The implants were left in their hosts for intervals varying from 3 to 76 weeks. Specimens from viable parts of the growing tumors were then removed for microscopic study.

Results

The presence of androgenic hormone proved to be the main factor responsible for the transformation of adenofibroma to fibroma, but for the change to occur the tumor usually had to be exposed to this hormone for 25 or more weeks. Thus, in 47 "androgen-present" rats (normal males and androgen-injected castrates) transformation to fibroma occurred in 28 or 59.6 per cent after a period of 25 or more weeks, but in 18 similar rats in which the tumor was exposed to the androgens for a period of 3 to 25 weeks the incidence of fibroma development was only 27.8 per cent. (Table I.) Although the fibroma-producing effect of physiological amounts of androgens is delayed it is quite abrupt after about 25 weeks and it is for this reason that the results are grouped under the headings of "3-25 weeks" and "25-76" weeks in Table I.

In the absence of androgenic hormone, adenofibroma only rarely underwent fibromatous change. Thus, fibroma developed in none of 37 "androgen-absent" rats (normal females, castrates, and estrogen-injected castrates) in the "3-25 week" group and in only 3 of 49 similar rats in the "25-76 week" group (Table I). The 3 instances of fibromatous change in the latter group occurred in 2 castrate males and 1 castrate female, and in each of these cases the tumor had grown very little, if at all, since implantation, suggesting that the unfavorable growth conditions had caused

* This investigation was aided by grants from the Thomas Brittingham Fund and the Jonathan Bowman Fund.

[1] Amniotin was supplied by Dr. John Anderson of E. R. Squibb & Sons.

[2] Testosterone propionate was supplied by Dr. Erwin Schwenk of the Schering Corporation.

epithelial atrophy but that the more resistant fibrous elements had not yet become nonviable.

That the fibromatous transformation produced by androgens is not due to inhibition of the tumor as a whole is indicated by two findings: first, the microscopic structure of the resulting tumor is that of an actively growing cellular fibroma, and second, the rate of growth of the tumors becoming fibromatous is as great as that of those retaining the adenofibromatous structure. Thus, the average diameter, at 26 weeks, of 19 tumors which had become fibromas in male or androgen-injected hosts was 3.5 cm., while the average diameter of 42 tumors which remained adenofibromatous was 3.2 cm.

Table I: Influence of Time and Endocrine Status on the Incidence of Fibromatous Transformation of Mammary Adenofibroma

Endocrine status	Interval: 3-25 weeks			Interval: 25-76 weeks		
	Number of rats receiving implants	Fibromatous transformation		Number of rats receiving implants	Fibromatous transformation	
		No.	Per cent		No.	Per cent
Androgen-present groups						
Normal males	12	4	33.3	33	19	57.6
Testosterone-injected castrate males	5	0	0.0	11	7	63.6
Testosterone-injected castrate females	1	1	100.0	3	2	66.6
Total: Androgen-present groups	18	5	27.8	47	28	59.6
Androgen-absent groups						
Normal females	26	0	0.0	23	0	0.0
Castrate females	..	..	..	6	1	16.6
Castrate males	2	0	0.0	13	2	15.3
Estrogen-injected castrate females	3	0	0.0	5	0	0.0
Estrogen-injected castrate males	6	0	0.0	2	0	0.0
Total: Androgen-absent groups	37	0	0.0	49	3	6.1

The tendency for the 11 different strains of adenofibroma to change to pure fibroma by the action of androgens varied from zero to 100 per cent. However, the tumors of each strain showed some relative increase in fibrous tissue after prolonged androgenic action. Therefore, while complete transformation to fibroma occurred in only 59.6 per cent after 25 weeks or more, a tendency for at least partial fibromatous change occurred in practically every instance.

Discussion

The fact that androgenic hormone stimulates rather than retards the epithelium of the normal rat mammary gland (5, 11) makes it difficult to understand how this hormone can cause the disappearance of the epithelium of mammary adenofibroma, a tumor which ordinarily responds like the normal gland to hormonal influences (6).

Since all the adenofibromas were originally derived from females, the possibility was considered that epithelium derived from female mammary glands might react to androgens in a manner different from that of epithelium from male mammary glands. To test this possibility, testosterone propionate in biweekly doses of 0.25 mgm. was injected into 4 castrate males and into 4 castrate females for 30 weeks. The result as shown by whole mounts and microscopic sections of the mammary glands was stimulation of mammary epithelium in the females in almost the same degree as in the males. There was no evidence of inhibition of the female mammary epithelium, and therefore the retardation of the epithelium of adenofibromas cannot be due to a sex difference in the response of mammary epithelium to androgens.

Another possible explanation of the transformation to fibroma by androgen is based on the differential response of the various mammary gland elements to the hormone. Thus, as Astwood and Geschickter (1) have pointed out, the androgens stimulate a characteristic type of acinar development with no stimulation of the duct tree itself. The fact that the epithelium of adenofibroma is largely of duct origin may well account for the lack of stimulation by androgens. However, there is no demonstrable inhibition of normal mammary duct epithelium by androgens and hence the inhibition of adenofibroma epithelium is not satisfactorily explained on this basis.

The possibility that androgens may cause a slight stimulation of the fibrous components of adenofibroma causing them to crowd out the epithelium was considered. However, since mammary glands which had been stimulated by androgens for long periods showed no tendency to fibrosis, this explanation is untenable. Moreover, the fact that androgens had little or no stimulatory effect on transplanted mammary fibroma (7) is further evidence against this explanation.

Therefore, at present no adequate explanation based on normal mammary physiology can be given for the

fibromatous transformation of adenofibroma by androgens. It would seem that in the process of becoming neoplastic the epithelium of rat adenofibroma becomes sensitive to an inhibitory effect of androgenic hormone.

That the effects of androgens are exerted upon the mammary structures through the mediation of the anterior pituitary gland is probable (9, 10), but since in these experiments the pituitary gland was always present it is permissible to speak of an androgenic effect upon the mammary gland even though it is conceded to be an indirect effect.

A clinical observation of interest is that benign mammary tumors in males tend to be predominantly fibrous while in females epithelial tumors predominate (2, 12). On the basis of the present experimental findings it may well be that the frequency of fibrous tumors in males is the result of the inhibition of the glandular components of fibro-epithelial tumors by androgenic hormone. Of some 400 benign mammary tumors studied in this laboratory all occurred in females with 1 exception and in this male the tumor was a pure fibroma.

While the complete obliteration of 1 component of a mixed tumor by a hormone is of fundamental interest, the phenomenon has no value in the treatment of adenofibroma since the remaining fibromatous component continues to grow unabated. There is the possibility, however, that the prolonged action of androgens upon pure adenoma of the mammary gland may produce complete regression of this tumor. Further experiments to test this hypothesis are in progress.

Summary and Conclusions

1. Androgenic hormone tends to transform mammary adenofibroma to pure fibroma in rats.

2. After 25 or more weeks of exposure to physiologic amounts of androgens fibromatous transformation of adenofibromas occurred in 28 of 47 rats (59.6 per cent) and partial transformation took place in the rest. Less than 25 weeks' exposure produced relatively little change in tumor histology.

3. Adenofibroma was never transformed to fibroma in females or in estrogen-injected castrates, and only rarely so in untreated castrates.

4. The fibromatous transformation produced by androgens does not depend upon inhibition of the growth of the tumor as a whole.

5. Different strains of adenofibroma vary in the degree to which they respond to the fibroma-producing influence of androgens.

6. No adequate explanation based on normal mammary physiology was found for the transformation of adenofibroma to fibroma. Only when the mammary epithelium is a part of a benign mammary tumor does it become sensitive to the inhibitory effect of androgenic hormone.

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Cancer in Hainan, China

A Supplementary Statistical Study of 451 Cases with Special Reference to Age, Anatomical Distribution, and Etiology

Nathaniel Bercovitz, M.D., F.A.C.S.

(From the American Presbyterian Hospital, Hoi How, Hainan, China)

(Received for publication November 16, 1940)

It is now 20 years since a preliminary report on cancer in Hainan was published (1). Since that time several attempts have been made to prepare a subsequent report, but various circumstances made this impossible. One set of records was destroyed during the disturbances caused by communist invasions. Several times medical work in Hainan was practically stopped because of extensive banditry and general lawlessness. At other times war conditions have kept people away from the hospital.

The present report is based on records of 451 cases, and presents a fairly accurate picture of cancer in Hainan as found during recent years. Of the cases reported, 373 were seen in the American Presbyterian Hospital, Hoi How; 48 were reported from the American Presbyterian Hospital, Kachek, by Dr. S. P. Seaton and Dr. E. Morse; 30 cases were reported from the American Presbyterian Hospital, Nodoa, by Dr. H. F. Burkwall and Dr. E. Morse.

TABLE I: AGE DISTRIBUTION OF CANCER CASES IN 1919 (1) AND 1939

	No. cases		Per cent	
Age	1939	1919	1939	1919
40 years and over	263	65	58.3	49.7
Under 40 years	188	66	41.7	50.3
Under 35 years	115	47	25.5	36.0
Under 25 years	53	25	11.7	19.0
Total number	451	131		

Most of the cases reported have come to operation. A serious defect in the report is the absence of record of many patients with inoperable cancers seen in outpatient clinics. Another serious defect is the inability to secure permission for autopsies in Hainan. However, more than half of the cases which have been operated on have had pathological examination of the tissues removed. This has been made possible through the courtesy of the Department of Pathology of the Peiping Union Medical College, which has been very generous in doing the work on material sent to them.

In the present study comparison is being made with the statistics reported 20 years ago. The comparison of the age groups for the two reports will be seen in Table I. It will be noted that while the incidence of malignant disease below the age of 40 is still high, the percentage of all cases reported is now 41.7 as against 50.3 per cent 20 years ago. One reason for this will probably be found in a study of Tables II and III. Table II presents the anatomical distribution as found 20 years ago. Table III shows the anatomical distribution as found in the present report and shows forms of cancer not found 20 years ago. Most of these

TABLE II: ANATOMICAL DISTRIBUTION OF CASES IN REPORT OF 1919 (1)

			Age		Sex	
Anatomical distribution	No. cases	Per cent	Over 40	Under 40	Male	Female
Penis	29	22.1	18	11		
Skin	29	22.1	16	13	23	6
Glands, neck	26	19.8	8	18	22	4
Face, scalp	21	16.0	9	12	17	4
Breast	10	7.6	7	3	2	8
Bone	8	6.1	3	5	6	2
Testicle	3	2.4	2	1	..	..
Anus	3	2.4	1	2	2	1
Tongue	1	0.7	1	0	1	0
Colon	1	0.7	0	1	1	0

are in the group of patients over 40 years of age. These include, among others, cancer of the cervix, stomach, ovary, eye, and nose. Cancer of the cervix, uterus, and ovary have been seen only within the past 5 or 6 years. There was a complete absence of cancer of the female genitalia in the 1919 report. The present report lists a total of 30 or 6.6 per cent of the total, and includes cancer of the ovary, uterus, cervix, vulva, and vagina. It is only within the past few years that gynecological cases have been coming for treatment at all, and probably more of these cases will be seen in the years to come. Few cases of uterine fibroids have been seen.

Cancer of the stomach has been observed within recent years. Pain in the region of the stomach has always been a common ailment of the Chinese here,

especially among men. Most cases were diagnosed as gastritis of one kind or another, or of ulcer; alcohol seems to play a large part in stomach distress here. However, within the past few years unmistakable cases of cancer of the stomach have been noted, all of them inoperable. This disease is undoubtedly more common than was formerly supposed.

TABLE III: ANATOMICAL DISTRIBUTION OF CANCER IN CASES OBSERVED FROM 1919 TO 1939

Anatomical distribution	No. cases	Per cent	Age: Over 40 years	Age: Under 40	Sex: Male	Sex: Female
Glands of neck	97	21.51	36	61	69	28
Breast	73	16.18	57	16	3	70
Skin	73	16.18	45	28	42	31
Penis	57	12.63	37	20	..	..
Bone	26	5.76	8	18	22	4
Cervix	15	3.32	9	6	..	..
Stomach	11	2.43	11	0	6	5
Ovary	10	2.21	7	3	..	..
Eye	10	2.21	5	5	6	4
Nose	8	1.77	5	3	5	3
Tongue	7	1.55	6	1	7	0
Lip	6	1.33	5	1	5	1
Parotid	6	1.33	2	4	3	3
Inguinal glands	6	1.33	3	3	6	0
Eyelid	5	1.10	3	2	3	2
Antrum	5	1.10	5	0	1	4
Testicle	5	1.10	3	2	..	..
Liver	4	.88	3	1	4	0
Abdomen (mesenteric)	3	.66	1	2	3	0
Uterus	3	.66	2	1	..	..
Rectum	3	.66	3	0	1	2
Brain	2	.44	1	1	1	1
Hodgkin's disease	2	.44	0	2	2	0
Spleen	2	.44	0	2	1	1
Intestines	2	.44	0	2	1	1
Ear	1	.22	1	0	0	1
Throat	1	.22	1	0	0	1
Sublingual glands	1	.22	0	1	1	0
Axillary glands	1	.22	0	1	0	1
Lungs	1	.22	1	0	1	0
Omentum	1	.22	1	0	0	1
Bladder	1	.22	0	1	1	0
Vulva	1	.22	1	0	..	..
Vagina	1	.22	0	1	..	..
Prostate	1	.22	1	0	..	..

It will be noted in Table III that the first 4 types of cancer listed, cancer of the glands of the neck, cancer of the breast, cancer of the skin, and cancer of the penis account for 300 or 66.6 per cent of the cases reported.

Cancer of the glands of the neck is the most frequent form of malignant disease, accounting for 97, or 21.51 per cent of the cases reported. In spite of forms of cancer in this report which were not observed in 1919, the percentage of cases of this form of cancer is higher than that of the 1919 report, which was 19.8 per cent. Most of these cases are in young people; that is, in the group under 40 years of age. Most of them occur in men, practically all of whom are farmers. Many of the cases are inoperable, even for palliative operation, when first seen. Many of the cases show discrete, movable glands, with no apparent sign as to primary location other than in the neck. We now feel that except for temporary relief from pressure, not much is gained by operating upon these patients. Unfortunately, radium and high voltage x-ray therapy are not yet available for treatment of malignant conditions in Hainan.

Microscopic diagnosis of these cases is usually "lympho-endothelioma," lymphosarcoma, or metastatic carcinoma.

While the gland cancers are generally considered to be metastatic, the location of the primary tumor presents a problem in the majority of the cases. There are patients whose symptoms and findings leave no doubt as to the location of the primary growth in the upper nasopharynx, or even in the brain. On the other hand, many cases of cancer of the glands of the neck are seen, with enormously enlarged, hard, fixed masses of glands on both sides of the neck, in some cases even with ulceration, in which it is impossible to locate a primary growth.

In this report are included 2 cases of Hodgkin's disease. It is interesting that among the many cases of cancer of the glands of the neck only 2 cases of Hodgkin's disease have been seen. These examples of a rare disease in China were reported in the *China Medical Journal* (2).

Inquiry from several sources has indicated that cancer of the glands of the neck is not seen commonly north of Canton and Hongkong. It does not even appear to be as common in Canton and Hongkong as in Hainan.

Just why cancer of the glands of the neck should be so common in Hainan is difficult to explain. None of the observed cases have been associated with cancer of the antrum, tongue, lip, or mixed tumor of the parotid. Impossibility of securing autopsies makes detailed study of these cases impossible. That so many of these cases are young farmers, men and women, cannot be dismissed lightly. Whatever the causative factor may be, it strikes early in life. It would seem that this condition calls for special study.

Cancer of the breast has risen from 7.6 per cent in the 1919 report to 16.18 per cent in the present report. This is in line with what would be expected as more women come to the hospitals for treatment. Unfortunately, most of the cases seen are advanced, with foul ulcerations and with gross involvement of the axillary glands.

The percentage of skin cancers is still high. No

attempt has been made to segregate these skin cancers into types. Cancer of the lip and eyelid have been classified separately. As in the 1919 report, most of the skin cancers are the result of previous injuries to the skin, either burns, cuts, or development on keloid formation. Although lower than the 1919 report, the percentage of skin cancer is much higher than found in America.

It will be noted that there is still a large proportion of cases of cancer of the penis. Although the present report of cancer of the penis is considerably lower than the 1919 report, the present figure, 12.63 per cent, is about 5 times the percentage found in America.

Recent observations have convinced us that phimosis is a primary etiological factor in cancer of the penis. Extreme phimosis is very common among the Hainanese, in many cases involving dense adhesions of the foreskin to the glans penis, with only a very small opening for urine to escape. Many adult cases come to the hospital for circumcision because of the irritation of the phimosis. For many years only advanced cases of cancer of the penis were seen, with complete ulceration and cauliflower growth of the glans and body of the penis, and with involvement of the inguinal glands. At that time caustics used because of venereal disease were supposed to bear a causative relation to the malignant disease of the organ.

However, the early cases seen within the past few years demonstrate the role which phimosis seems to play in these cases. No early case of cancer of the penis without phimosis has been seen. There seems to be no relation to syphilis or other venereal disease in cancer of the penis. Few of the cases show positive Kahn tests, and most of the cases are farmers from districts, far from the cities, where the incidence of venereal disease is very low.

Several cases have come to the hospital with complaint of a nodule within the phimotic foreskin. At operation there has been found an early epithelioma, confirmed by microscopic sections, on the mucous surface of the skin near but not on the glans penis. Apparently, the involvement of the glans comes from extension of this primary growth.

In general the findings in Hainan are similar to those reported by Ngai (3). Even admitting the presence of phimosis as an etiological factor in this disease, the fact that cancer of the penis occurs in such a large proportion of all cases seen, a figure far above that of other countries where phimosis is also common and also untreated, would seem to indicate that there is some additional factor which is responsible for the prevalence of this disease in Hainan. As in the case of cancer of the glands of the neck the fact that so many of these cases occur in farmers seems to be significant. Another point of interest is the fact that of the 30 cases reported from Nodoa by Dr. Burkwall and Dr. Morse, no cases of cancer of the penis are included. The series is admittedly small, but it covers a period of about 4 years, and would seem to be typical of the findings of that region. Geographically, Nodoa is on the western side of Hainan, in the hills; and racially, Nodoa is the center of numerous tribes of aboriginal and semi-aboriginal peoples, with very few Hainanese in the region.

Most of the tumors of bone were osteosarcoma. Most of the patients were seen too late for treatment, but even in the early cases amputation was refused.

In this series only 3 cases of cancer of the rectum are reported. Lymphogranuloma of the rectum with stricture is seen occasionally. Syphilis among the Hainanese is common. Rectal disease, hemorrhoids and fistula *in ano,* are extremely common in Hainan. Dysentery, amebic and bacillary, is also common. The relative absence of cancer of the rectum is therefore of interest.

One case of cancer of the prostate was seen, which did not come to operation. In general, enlargement of the prostate is not nearly as common as in the United States. In a few cases in old men, usually alcoholics with previous history of gonorrhea, retention of urine because of enlarged prostate has been seen.

Cancer of the esophagus has not been observed. Cancer of the larynx has not been seen, although tuberculosis of the larynx, representing the terminal stage of pulmonary tuberculosis, is very common in Hainan. Only 1 case of cancer of the lungs has been noted. In spite of the prevalence of leprosy in Hainan no case of cancer of any kind among lepers is recollected. In a personal communication, Dr. James L. Maxwell of Shanghai, who for many years did extensive work among the lepers of China states that he cannot remember having seen a case of cancer among lepers. Further information on this subject is now being gathered.

The picture of cancer in Hainan shows that 34.14 per cent of 451 cases are cancer of the glands of the neck or cancer of the penis. This figure is out of all proportion to the incidence of these 2 kinds of cancer elsewhere. Furthermore, the absence of cancer of the esophagus and larynx, and the practical absence of cancer of the rectum, prostate, and lungs, would seem to be significant. Further statistical studies will show some changes from the figures reported; and when it is possible to perform autopsies much more accurate information will be obtained.

As these variations in the statistics of cancer as found in Hainan are considered, it must be remembered that the whole incidence of diseases in Hainan differs from the incidence in other parts of the world.

For example, except for a few imported cases diphtheria is almost unknown. Scarlet fever has not been found in Hainan. Very few cases of appendicitis occur there. It is only within recent years that much typhoid fever has been seen in Hainan. In spite of the frequency of gonorrhea in men and women, surprisingly few cases of stricture or salpingitis are seen, although gonorrheal arthritis occurs. Other forms of arthritis are seen, but they are not very common when compared to this disease in America. Acute articular rheumatism is almost never observed. It should also be noted here that in spite of the frequency of syphilis, general paresis is unknown in Hainan. Most of these diseases are seen in other parts of China.

On the other hand, malaria and hookworm disease are almost universal in Hainan. Dysentery is common. There are epidemics of bubonic plague, cholera, and small pox. Leprosy and tuberculosis are common. Rickets is almost never seen. Beriberi and keratomalacia are frequently seen. Stone in the urinary bladder is very common although only 1 case of renal calculus has been diagnosed. Also, only 1 case of cancer of the bladder and no cases of cancer of the kidney have been noted.

Further variables in Hainan are the diet of the people here, and the fact that generally speaking their blood pressure is lower than for similar age groups in America. Diabetes is not seen often, and then only among well-to-do men who indulge freely in heavy eating and drinking. These men are also usually the ones who show high blood pressure. Incidentally, eclampsia is very seldom found. In a period of 2 years in which 1654 maternity cases, involving many abnormal ones, were registered in the hospital, only 1 case of eclampsia was seen.

The unusual incidence of disease in Hainan, so different from other parts of the world, makes this statistical study of cancer important. Hainan is an isolated portion of China, a large island, off the southernmost part of China, in the tropics, with geographical, anthropological, and disease conditions differing from the mainland and from many other lands. The study of masses of facts from different parts of the world, with different races, and disease conditions, and the correlation of these findings are all necessary to a broad study of cancer.

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An Abortifacient Substance in Human Urines*†

Maurice Ross, M.D., and Ralph I. Dorfman, Ph.D.

(From the Laboratory of Physiological Chemistry and the Adolescence Study Unit, Yale University School of Medicine, New Haven, Conn.)

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The work of Elsasser and Wallace (2), which has shown that the serum and the urine of patients suffering from cancer can cause pregnant rabbits to abort, has opened interesting avenues for research. These same workers also found that the urines and serums of cancer patients produced necrosis of Walker carcinoma 256 which had been grafted into rats. In view of these observations it seemed desirable to attempt to concentrate this abortifacient substance and to investigate its nature.

The only information available as to the chemical nature of the hypothetical agent in the urine and serum of cancer patients which produces abortion in pregnant rabbits was that it is destroyed upon heating to 70°C, and that it disintegrates upon standing at room temperature for over 24 hours (3). On the basis of these facts it seemed likely that this material might be a protein. Therefore, attempts at concentration were made by a method previously used for the precipitation of protein. Fortunately, methods which had been employed for obtaining gonadotropic hormones from the urine were capable also of precipitating small amounts of proteins. The method employed was shown to concentrate material possessing lactogenic activity [1] in addition to the gonadotropic material (17). Therefore, the possible abortifacient action of both the gonadotropic substance and prolactin, as well as of some nonspecific protein substances, as egg albumin and peptone, was investigated.

Subjects, Materials, and Methods

Patients in whom the diagnosis of a malignant tumor was beyond all reasonable doubt were selected. Four males between the ages of 18 and 31 years who were apparently in good health and 3 female patients on the medical service of the New Haven Hospital who, as far as could be ascertained, had no malignant growths at the time, served as noncancerous controls. The final diagnoses are indicated in the accompanying tables.

With 2 exceptions all the cancer patients had malignant tissue in their bodies at the time that the urine specimens were collected. Patients A. D. and L. E. who had had carcinoma of the breast without lymph node metastases, had their cancers removed before the urine samples were collected.

The 24-hour urine specimens were preserved with toluene. Chemical purification was begun within a few hours after the completion of the collection, except for a few specimens which were kept in the refrigerator for about 24 hours. The method of concentration used was that previously described by Freed and Hecter (7) with modifications. It consisted, in brief, of the following processes:

1. Twenty cc. of 10 per cent sodium tungstate were added to each liter of urine at a pH of about 3.
2. The precipitate thus formed was washed 3 times with a 3 to 1 ethanol-ether mixture.
3. The sediment was then extracted with a 60 per cent acetone-4 per cent ammonia mixture.
4. The concentration of acetone in this mixture was increased to 80 per cent to form a precipitate.
5. This precipitate was dissolved in 50 per cent acetone and again precipitated by increasing the concentration of acetone to approximately 80 per cent and adding a few drops of acetic acid.
6. The product was washed successively in 80 per cent ethanol, 95 per cent ethanol, and 99 per cent acetone, then dried in a vacuum desiccator to yield the final concentrate used in these experiments.

The concentrates were kept in the form of powders until the time of injection, when they were dissolved in a saline solution formed by adding N/10 sodium hydroxide and neutralizing with N/10 hydrochloric acid. Egg albumin, prolactin, and peptone were dissolved in saline by the same procedure. The egg albumin was a preparation which had been twice crystallized and dialyzed sulfate-free. The preparation of prolactin had 90 per cent of the activity of crystalline prolactin. The peptone was a commercial product made from dried meat by Merck & Co., Inc.

Gonadotropic substances from 2 sources were used. One preparation, apoidin, was a chorionic gonadotropin prepared from human pregnancy urine by Parke, Davis & Co. Each cc. of solution contained

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† This paper is based upon a thesis submitted by Maurice Ross to the Faculty of the School of Medicine, Yale University, June 1940, in candidacy for the degree of Doctor of Medicine.

[1] This lactogenic activity was demonstrated by both the local intradermal method and the systemic procedure.

500 rat units of gonadotropic activity and 5.2 mgm. of total solids; the other, gonadin, from the Cutter Laboratories of Berkeley, Calif., was a concentrate of pregnant mare serum containing 200 Cole-Saunders units and 1.789 mgm. of total solids per cc. According to assays on mice, Dr. H. R. Catchpole estimated 200 Cole-Saunders units to be approximately equivalent to 500 rat units. In order to give small doses, the preparations were diluted with distilled water.

The animals used in these studies came from a single colony of white mice and were fed on adequate amounts of Tioga dog pellets, oats, and lettuce. All breeding females were examined daily for the presence of vaginal plugs. The day on which the plug was found was counted as the 1st day of pregnancy. To be certain that the mice were pregnant they were examined from the 9th to 13th days for the appearance of the placental sign. Only mice on which these 2 phenomena were observed were used. The placental sign appeared between the 9th to the 13th day of pregnancy. In 1 case it was present on the 14th day. Seven of the animals were injected on the 16th, 17th, and 18th days of pregnancy; the rest on the 15th, 16th, and 17th days. Three subcutaneous injections were given at 24-hour intervals, so that each animal received 3 injections in the course of 48 hours unless it expelled its fetuses or died in a shorter period.

Results

The results obtained with concentrates of cancer and noncancer urines are given in Tables I and II, respectively. Resorption was considered to have occurred in those mice in which pregnancy was diagnosed by the 3 criteria of vaginal plug, placental sign, and appearance but which ceased to appear pregnant and delivered no young during at least a week beyond the expected date of parturition. Some mice were sacrificed and autopsied a few days after the expected date of parturition without any evidence of fetuses in the uterus. Other mice expelled dead fetuses within 72 hours after the 1st injection. In the columns indicating the number of animals that expelled their fetuses and the number of pregnant mice that died, the approximate time elapsing between the 1st injection and the expulsion or death is given in parentheses. A normal delivery is one in which litters were delivered on the 19th to 21st day of pregnancy.

The urinary concentrates prepared from the urines of all 7 cancer patients contained material capable of producing resorption or expulsion of the young or both. Resorption was noted after the administration of 1/26 to 1/12 of the total 24-hour concentrate (Table I).

Table I: Effect of Concentrates of Cancer Urines on Pregnant Mice

Patient and diagnosis	Sex	Age	Fraction of 24-hours sample injected daily	Weight of concentrate injected daily (mgm.)	No. of mice injected	No. of resorptions	No. of expulsions	No. of mice that died	No. of normal deliveries
L. D. Cancer of lung	Male	37	1/43	7.5	4	0	0	0	4
			1/22	14.6	4	2	0	0	2
			1/14	8.4	2	2	0	0	0
L. F. Cancer of colon	Female	32	1/24	9.5	2	1	1 (24 hrs.)	1 (24 hrs.)	0
			1/12	19.0	2	0	1 (24 hrs.)	2 (48 hrs.)	0
R. Z. Cancer of vulva	Female	72	1/26	5.0	1	1	0	0	0
			1/13	10.0	2	2	0	0	0
			2/13	20.0	2	0	2 (24 hrs.)	0	0
S. K. Cancer of nasopharynx	Male	53	1/24	4.1	2	0 or 1?	0	0	1 or 2?
			1/12	8.2	2	1	1 (72 hrs.)	0	0
A. D. Cancer of breast	Female	46	1/24	1.2	1	0	0	0	1 *
			1/12	2.4	2	2	0	0	0
C. R. Cancer of stomach	Female	70	1/16	2.2	3	2	1 (48 hrs.)	0	0
L. E. Cancer of breast	Female	44	1/16	4.2	4	1	0	0	3

* Litter of 3 dead fetuses.

The urine of patient C. R. yielded the most active concentrate on a weight basis. As little as 2.2 mgm. per day for 3 days caused resorption in 2 of 3 animals; the 3rd animal expelled her young 48 hours after the 1st injection.

Of the 3 normal men studied (Table II) the urine of only 1 individual was found to contain abortifacient material. In the instance of M. R. resorption was noted when the equivalent of 1/12 of a 24-hour sample was administered. The urinary concentrates of the other 3 men failed to show abortifacient action at levels of 1/12, 1/12, and 1/16, respectively.

Definite evidence for resorption and expulsion was found when the urinary concentrates of 3 noncancerous but otherwise ill women were administered to preg-

nant mice. In 1 case the equivalent of 1/12 of the daily urinary output was active in producing expulsion, while resorptions were noted in the other 2 cases at the same dosage level.

Table III shows the effect on pregnant mice of injections of crystalline egg albumin, prolactin, and peptone. At dosage levels of 10, 20, and 36 mgm. of egg albumin respectively, no abortifacient action was noted. However, when the concentration of egg albumin was increased to 45 mgm. per injection, expulsion was noted. Prolactin produced abortifacient effects when administered at 8 and 10 mgm. dosage level. The administration of 10 mgm. of peptone per day produced expulsion in 3 of 6 animals while 5 mgm. of peptone was inactive.

The results obtained with mice injected with the gonadotropic substances are grouped in Table IV. For the purpose of tabulating the results, it is assumed that 2 Cole-Saunders units are the equivalent of 5 rat units. The 21 pregnant mice that received 3 doses of 7.5, 15.0, and 25.0 rat units of gonadotropic substances produced 89 dead fetuses and only 1 live newborn. Those mice which received 3 injections of 3.75 rat units of apoidin had 24 dead fetuses and 6 live newborn. None of the young survived more than 24 hours. Some of the dead fetuses were delivered as late as the 24th day, other dead fetuses were found in the uterus on autopsy after the expected day of delivery.

In a mouse given 3 doses of 15 rat units of gonadin and in another given the same number of doses of 7.5

TABLE II: EFFECT OF CONCENTRATES OF NONCANCER URINES ON PREGNANT MICE

Patient and diagnosis	Sex	Age	Fraction of 24-hours sample injected daily	Weight of concentrate injected daily (mgm.)	No. of mice injected	No. of resorptions	No. of expulsions	No. of mice that died	No. of normal deliveries
R. D. Normal	Male	28	1/24	1.8	2	0	0	0	2
			1/12	3.7	2	0	0	0	2
S. B. Normal	Male	18	1/24	2.0	2	0	0	0	2
			1/12	4.0	2	0	0	0	2
M. R. Normal	Male	25	1/12	2.5	2	1	0	0	1
			1/8	3.8	1	1	0	0	0
			1/24	1.5	1	0	0	1 *	0
			1/12	3.1	2	1 †	0	0	1
A. W. Normal	Male	31	1/16	2.2	3	0	0	0	3
E. O. Syphilis of central nervous system; fibroadenoma of breast.	Female	36	1/24	3.2	1	0	1 (72 hrs.)	0	0
			1/12	6.4	2	0	2 (24 hrs.)	0	0
S. I. Arteriosclerosis, heart disease.	Female	53	1/24	5.8	2	0 or 1?	0	0	1 or 2?
			1/12	11.7	2	2	0	0	0
F. S. Chronic tonsilitis, sinusitis, otitis.	Female	51	1/24	1.9	2	0	1 (24 hrs.)	0	1
			1/12	3.8	2	2	0	0	0

* Died on 21st day, still pregnant.
† Died on 25th day.

TABLE III: EFFECTS OF EGG ALBUMIN, PROLACTIN, AND PEPTONE ON PREGNANT MICE

Substance injected	Injected daily (mgm.)	No. of mice injected	No. of mice that resorbed litters	No. of mice that expelled litters	No. of mice that died	No. of mice with normal deliveries
Egg albumin	10	4	0	0	0	4
Egg albumin	20	2	0	0	0	2
Egg albumin	36	3	0	0	0	3
Egg albumin	45	2	0	1 (24 hrs.)	0	1
Prolactin	5	5	0	0	0	5
Prolactin	8	3	2	0	0	1
Prolactin	10	5	2	2 (24 hrs.)	0	1
Peptone	10	6	0	1 (24 hrs.) 1 (48 hrs.) 1 (72 hrs.)	0	3
Peptone	5	7	0	0	0	7

TABLE IV: EFFECT OF GONADOTROPIC SUBSTANCES ON PREGNANT MICE

Substance injected	Rat units * injected daily	No. of mice injected	No. of mice that delivered live litters	No. of mice that delivered live and dead fetuses	No. of mice that delivered dead litters	No. of mice with delay of delivery	No. of mice that resorbed litters
Gonadin †	25.0	4	0	1	1	2	0
Apoidin ‡	25.0	4	0	0	1	1	2
Gonadin	15.0	5	0	0	1	3	1
Apoidin	15.0	5	0	0	0	4	1
Apoidin	7.5	3	0	0	1	2	0
Apoidin	3.75	6	1	1	2	2	0

* For the purpose of tabulating the results with the gonadin injections, it is assumed that 2 Cole-Saunders units are equivalent to 5 rat units.
† Gonadin is a concentrate of pregnant mare serum prepared by the Cutter Laboratories.
‡ Apoidin is a chorionic gonadotropin prepared from human pregnancy urine by Parke, Davis & Co.

rat units of apoidin, the uteri ruptured and 1 of the fetuses in each case was found free in the peritoneal cavity.

Resorption was demonstrated when 3 doses of either 15 or 25 rat units of gonadotropic hormone were administered.

Discussion

Since the urinary preparations injected contained both prolactin and gonadotropic substance, endocrine factors in abortion must be carefully considered. On the basis of assays done by Dr. A. White on some of the preparations, it appears unlikely that any 1 of the doses injected contained as much as 5 mgm. of prolactin. Although larger amounts of prolactin have caused abortion, 3 daily doses of 5 mgm. each given in the routine manner did not produce abortion in any of 5 mice. According to Katzman (11), Friedman and Weinstein (8), Drips and Osterberg (1), and Heller and Heller (10), it is extremely unlikely that any of the patients excreted more than 100 rat units of gonadotropic hormone per day. On that basis none of the animals received more than 3 doses of 8.33 rat units each. Doses of gonadotropic hormone as low as 3.75 rat units on 3 successive days prevented delivery of normal litters, but the action of this hormone differs from that observed with the urine extracts. The gonadotropic substances cause the retention of the litters in the uterus beyond the expected date of delivery and death of the fetuses. These results are similar to those observed by Snyder (14), who prolonged pregnancy in multiparous rabbits by the inhibition of parturition at term by the administration, 25 days after mating, of a single intravenous injection of 40 rat units per kilogram of extract of urine of pregnant women (antuitrin S, Parke, Davis & Company). Such an action was not observed with urine extracts, egg albumin, peptone, or prolactin; on the other hand, while large doses of these substances produced expulsion of dead fetuses and sometimes death of the mother within 72 hours after the 1st injection, similar results were never observed with the gonadotropic preparations. External evidence of the action of the gonadotropic hormones was never observed until at least 4 days after the 1st injection.

In view of work done by Flaks and Ber (6), it seems appropriate to discuss gonadotropic antihormones at this point. Using the inhibition of estrus as their criterion, these workers observed antigonadotropic activity in 81 per cent of urines of cancer patients and in 27.6 per cent of urines of persons with other diseases, exclusive of disease of the urogenital system, but no such activity was found in normal urines. Thompson (15) found gonadotropic antihormones capable of terminating pregnancy in dogs. Thus it seems possible that we may be dealing with a substance similar to that described by Flaks and Ber and by Thompson.

Since our preparations were washed 3 times with a 3:1 ethanol-ether mixture, practically all of the estrogens were removed. Although Rawson and Starr (12) claimed to have found slight degrees of thyrotropic activity in urine, Emerson and Cutting (5) were not able to demonstrate any urinary thyrotropic activity in normal men and found it only rarely in patients with hyperthyroidism and hypothyroidism. Upon assaying 1 of our preparations by the day-old chick method of Smelser (13), Dr. A. White obtained a negative response with 5 mgm. On the basis of these findings, it is improbable that thyrotropic substance was responsible for the abortifacient action observed. Since the oxytocic factor works by directly stimulating uterine musculature and thus is not capable of producing resorption, its fundamental action is different from that observed in these experiments.

To prevent a possible effect of infections and general diseases on the results, any animal that showed signs of illness was eliminated. To rule out pelvic disease as a factor, autopsies were performed on all animals in whom there was any reason to suspect an abnormality. Uterine tumors were found on 3 occasions and the animals in which they were found were omitted from the experiments.

Chemical agents may play an important role in producing abortion, but it does not seem probable that the process of treatment introduced any new chemical agents that might have this effect, since similar results were obtained by Elsasser and Wallace (2), Thompson, Hale, and Whitcomb (16), and Gershon-Cohen, Samson, and Shay (9) using untreated urine in rabbits. Furthermore, the only added reagent not normally present in urine, except for solvents, which were completely removed before the final product was obtained, was sodium tungstate. Another factor which makes it improbable that the method of treatment of the urine introduced toxic agents is that some of the preparations did not exhibit abortifacient action. Substances such as 1,2,5,6-dibenzanthracene, 3,4-benzpyrene, and 1,2-benzanthracene, which Wolfe and Bryan (18) have shown to produce placental hemorrhage and resorption of fetuses in rats, if present at all, were carefully removed from the preparations by washing 3 times with 3:1 ethanol-ether mixture. The material which was used for injection contained no solvents other than saline.

The purpose of the egg albumin injections was to rule out a nonspecific protein reaction as the cause of the abortions. Whereas extracts of urine in quantities as low as 2.2 mgm. in each of the 3 doses were found capable of producing resorption and expulsion, 3 injections of 36 mgm. of egg albumin each did not affect the course of pregnancy in mice.

Since it is realized that impure protein products may have greater toxicity than crystalline egg albumin, peptone was administered to a series of animals. In view of the fact that 3 injections of 10 mgm. of peptone produced premature expulsion of the fetuses in 3 instances while they failed to interrupt pregnancy in 3 others, and that as many injections of 5 mgm. of peptone per mouse did not interfere with pregnancy in 7 animals, the critical level above which all pregnant mice will abort and below which none will abort must lie between 5 and 10 mgm. of peptone. It seems fairly reasonable, therefore, to believe that in those cases where the dry weight of the urine extract given in a single dose was considerably less than those levels, the abortifacient action cannot be attributed to peptone alone.

The mice that aborted were kept in the same physical environment as the control mice that did not abort. The animals were never subjected to anesthesia. No procedures other than injections and necessary inspections were carried out. Four mice treated in a similar manner, except that saline alone was injected, did not abort or resorb the fetuses.

Summary and Conclusions

Protein-like substances were concentrated from the urines of 6 men and 8 women by a method which consisted of precipitation of the fresh urine with 10 per cent sodium tungstate, dissolving the precipitate in a 60 per cent acetone-4 per cent ammonium hydroxide mixture, and precipitating the desired fraction from this solution by increasing the concentration of acetone to about 80 per cent and adding a few drops of acetic acid. These concentrates were tested for abortifacient action by subcutaneous injection in pregnant albino mice.

The urine concentrates of every one of the 7 patients suffering from cancer exerted some abortifacient action. Concentrates of urine from 3 normal men similar to those used for cancer patients, gave no evidence of abortifacient action. In the urine of the other normal man some evidence of abortifacient action was present. The urine of all 3 of the noncancerous but otherwise ill women showed as much evidence of abortifacient material as did that of the cancer patients.

Egg albumin in a saline solution injected subcutaneously in larger doses by dry weight than those of the urinary powders did not have the same action. In doses of 5 mgm. a prolactin preparation which was approximately 90 per cent pure did not interfere with pregnancy in mice. Three subcutaneous injections of 8 mgm. each, however, were able to cause resorption, and with doses of 10 mgm. each it was possible to demonstrate both resorption and expulsion of the fetuses. Three daily subcutaneous injections of 10 mgm. of peptone were followed by normal deliveries in 3 pregnant mice and by premature expulsion of the fetuses in 3 others. Three doses of 5 mgm. of peptone each did not interrupt pregnancy in 7 mice. The action of gonadotropic preparations, in doses ranging from 3.75 to 25.0 rat units per day for 3 days, differed considerably from that of the urine extracts, egg albumin, peptone, and prolactin. The gonadotropic substances used in these experiments tended to produce death of the fetuses and their retention in the uterus beyond the expected date of delivery.

The authors are grateful to Dr. E. Allen, Dr. H. R. Catchpole, Dr. A. White, and Dr. A. W. Oughterson for their kind advice and cooperation.

Addendum.—After this paper had been submitted for publication, Ely (4) reported "the preparation of a fraction from the urine of patients with advanced cancer which causes resorption in pregnant rats and mice." He also found this material in the urine of a healthy adult male but in a much smaller concentration. These results are essentially in agreement with those obtained by us.

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Communications

Notes on the Current Literature of Cancer Research.

E. L. Kennaway

(From the Chester Beatty Research Institute, The Royal Cancer Hospital (Free), London, S. W. 3, England)

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At the present time, when definite progress is being made upon several lines in cancer research, in spite of the difficult conditions of war, it is well to consider how the data which are being obtained may be made available to all investigators with the greatest completeness, rapidity, and economy of effort. Such distribution of knowledge is the function of abstract journals, and the following notes refer to some difficulties which arise in abstracting the literature of cancer research.

I. The manifold aspects of the cancer problem cause the relevant papers to be distributed over a very large number of journals. Three instances of this scattering may be given.

A. If one considers only literature published in the British Empire, some 60 journals and annual publications would have to be examined in order to be reasonably sure that everything of any value having direct reference to cancer research had been found.

B. Three reports on the literature relating to "Chemical Compounds as Carcinogenic Agents" have been contributed to the *American Journal of Cancer* from this Institute. These deal with one part only of the subject of cancer, and they contain references to 871 papers in 114 journals. Of course journals dealing with chemistry were especially well represented, but the following classification shows that these did not form a large fraction of the total:

7	Journals dealing with cancer only.
81	Journals dealing with biology and medicine.
15	Journals dealing with chemistry and physics.
11	General scientific and institutional journals; e. g., *Nature, Proc. Roy. Soc.*
114	

C. An admirable summary of the literature up to 1936 relating to induced lung tumours in mice by Andervont (Pub. Health Rep. **52**:212. 1937) gives references to 30 papers in 14 different journals. The subject of induced lung tumours in mice, though of great importance in cancer research, is admittedly, from a more general point of view, a small one, and one may be inclined to ask whether the literature of such a subject could not by some means be concentrated into less than 14 journals? (No doubt in former days a German publisher could have inflicted upon the librarians of the world, and made highly profitable to himself, an *Archiv für die gesamte Induziertemäuselungengeschwülstenkunde,* or something of that sort).

Although the more out-of-the-way journals may not often yield anything of importance to cancer research it is obviously essential that they should be examined by abstractors. Every cancer research worker knows that he must look at such journals as the *Journal of Experimental Medicine,* or the *Journal of Pathology and Bacteriology;* the especial function of the abstract journal is to sift all the other literature which the individual worker cannot possibly examine. One would like to advocate a close watch upon various official and government publications; these may appear at very irregular intervals, and are difficult to hear of, sometimes difficult to obtain, and are often bulky and inconvenient in form, and expensive. Hence they are rather inaccessible, but some of them, such as the publications of the Registrar General for England and Wales, contain unique data about cancer which are of great interest and value.

As an example of the material of great importance to cancer research workers which may appear in the less widely read journals one may quote the work of E. P. Snijders and M. Straub who were the first to show, upon a proper statistical basis, that the organ incidence of cancer might be very different in two countries (in this case Sumatra and Holland) in which the total incidence was about the same. Their results were brought forward, first in the Dutch language in *Geneeskundig Tijdschrift voor Nederlandsch-Indië* in 1922 and subsequently in English in *The Transactions of the Fifth Biennial Congress,* Far Eastern Association of Tropical Medicine, Singapore, 1923.

II. The literature of cancer research would be lightened if the whole subject of surgical technique in relation to cancer, and the nursing of cancer patients, which is of course of the utmost practical importance, could be left to other journals. Thus a recent abstract in a cancer journal deals with the question of the

washing-out of the colon before operation on rectal cancer. This has nothing to do with cancer research. It is very doubtful whether surgeons, in this country at any rate, go to cancer abstract journals to learn about surgical technique, and certainly no other readers of such journals require this material. Again, a great deal of the literature upon the empirical treatment of cancer by radiations, as distinct from the use of radiations in research, could be omitted. But of course any isolation of the literature of laboratory cancer research from that of clinical cancer research is very undesirable, for both types of investigation are necessary for the study of cancer as it occurs in man. There is much clinical research to be done upon what has been well called the natural history of human cancer; *e.g.*, the large differences in the incidence of cancer of the upper alimentary tract upon the different social classes.

III. An evil which cancer research has to bear together with other branches of medical science is the enormous bulk of post-mortem reports. It appears to be a tradition of some pathologists of the post-mortem room that every relevant and irrelevant detail of an autopsy must be described fully. If a dozen or so of such reports can be collected and labelled Case 1, Case 2, and so on, the composition of a paper is greatly facilitated. Much of the information recorded is useless. Thus one may be told that an organ "was of a pale greenish-blue colour and slightly deliquescent in consistency." An appreciable part of the immense burden which the medical libraries of the world have to support upon their shelves must consist of such material.

Many of the lengthy papers on intracranial tumours which are abstracted in cancer research journals contain accounts of pressure symptoms which would be produced by any object within the skull and have nothing to do with cancer.

IV. For various reasons, some probably of a personal nature, papers are published in journals in which no one would expect to find them, for the subject of the paper is not comprised within the subject of the journal as expressed in its title. Thus much of the literature of the physiology of oestrogens has appeared in journals nominally devoted to anatomy, and a paper on induced cancer which does not contain a single word referring to radiology in any form may appear in a radiological journal. (The *Public Health Reports* of the United States Public Health Service has long been known to cancer research workers as one of their most valuable journals, so that in this case the incongruity of the title ceased to matter.)

V. Japanese journals in recent years have tended to publish an increasing number of papers wholly in Japanese, without any summary in any European language.[1] This literature is thus rendered practically inaccessible to readers in other countries who have not the help of a Japanese interpreter.

[1] It is somewhat ironical that the pioneer experiments of Tsutsui (Gann, **12**:17-21. 1918) on the production of cancer by application of tar to the skin of the mouse, a method which was soon adopted all over the world, were performed "mittelst eines an der Spitze stumpf abgeschnittenen japanischen Schreibpinsels," that is, by the instrument of that script which has done more than anything else to isolate the Eastern from the Western world.

Incidentally, one may note that Tsutsui's paper is even now well worth reading or re-reading. In five of the small pages of *Gann*, he gives, in a summary in German of his paper in Japanese, an admirably clear and concise account of the phenomena of the tar-painted mouse; indeed, in so far as purely observational methods are concerned, little of importance could be added to his description today. The number of important facts which Tsutsui learned from his first series of 35 tumour-bearing mice is remarkable. Thus in his description of the microscopic structure of the tumours he notes (1) that the down-growing cancer penetrates the layer of muscle beneath the mouse's skin, a process which later workers have used as a criterion of malignancy; (2) that metastases may occur in the lung; (3) that the tumour may resemble a spindle-celled sarcoma; and (4) that the growth of the tumour, when once well established, progresses whether painting be continued or not.

Abstracts

Abstracts are grouped under the following main headings: Reports of Experimental Research, Comparative Oncology, Clinical and Pathological Reports, Statistics, and Cancer Control and Public Health. Subheadings are used in accordance with the subjects of papers abstracted.

The initials of the abstractor are placed at the end of each abstract. Contributors of abstracts published in this issue are: S. Bayne-Jones, W. J. Burdette, P. P. Cohen, H. G. Crabtree, Marie Duran-Reynals, W. U. Gardner, A. Haddow, I. Hieger, E. L. Kennaway, A. Kirschbaum, Jean LeCompte, A. A. Liebow, R. R. Ludford, J. L. Melnick, C. A. Pfeiffer, L. L. Waters, and H. Gideon Wells.

REPORTS OF EXPERIMENTAL RESEARCH

Carcinogenic Compounds

AUERBACH, C. (Inst. of Animal Genetics, Univ. of Edinburgh, Edinburgh, Scotland.) Tests of carcinogenic substances in relation to the production of mutations in Drosophila melanogaster. Proc. Roy. Soc. Edinburgh, 60[2]:164-173. 1940.

The somatic mutation theory is accepted as the most plausible explanation for cancer, the strongest support for this being the parallelism between the effect of both x-ray and ultraviolet light on mutation and cancer. If a parallelism between mutation and cancer production by carcinogenic hydrocarbons could be established, the mutation theory of cancer production would be strengthened. These experiments were designed to detect any effect of carcinogens on the mutation rate in *Drosophila melanogaster*. Preliminary experiments in which carcinogens were fed yielded negative results. Adults and larvae were used, the former with greater success. Methylcholanthrene crystals were inserted mid-dorsally, and colloidal solutions of 1,2,5,6-dibenzanthracene (0.3%) and 9,10-dimethyl-1,2-benzanthracene (0.06%) suspended in Ringer's solution as given by Ephrussi and Beadle (Am. Nat., **70**:218-225. 1936) minus calcium plus 0.5% gelatine were injected mid-dorsally and laterally. The incidence of sex-linked lethals was taken as a criterion of the mutation rate. These were detected by the ClB method in the males. Females injected were heterozygous for *sc*^{s}*w*^{a}*bb* and *sc δ49 v od car*. They were crossed to *v* B^{M1} males, and the female F_1 were individually mated. The absence of *w*^{a} and *od* males in the F_2 progeny was taken as indicating the presence of a lethal in the treated or control female. No significant increase in mutation rate was found when single or multiple doses were used. The average amount injected was less than 0.2γ. There was a sex difference in the spontaneous mutation rate in the stocks used. The author accepts Sacharov's results of treating *Drosophila* eggs with methylcholanthrene as being encouraging but not significant. There are no tables or figures in the paper.—W. J. B.

BADGER, G. M., J. W. COOK, C. L. HEWETT, E. L. KENNAWAY, N. M. KENNAWAY, R. H. MARTIN, and A. M. ROBINSON. (The Chester Beatty Research Inst., The Roy. Cancer Hosp. (Free), London, England.) The production of cancer by pure hydrocarbons. V. Proc. Roy. Soc. s. B., 129: 439-467. 1940.

Tests for carcinogenic activity have been carried out with about 70 compounds, which are mostly new substances synthesized for this purpose and related to known carcinogenic compounds. The compounds tested were derivatives of 1,2-benzanthracene, cholanthrene, 3,4-benzpyrene, 3,4-benzphenanthrene, acridine, and fluorene. These were given by application to the skin, by injection *sub cutem*, and in some cases by the mouth. The results support the view that there is a definite association between molecular structure and carcinogenic action. Thus in the 3,4-benzphenanthrene series, which has not hitherto been investigated extensively, positions 1 and 2 seem to be positions for substitution which are more favorable than are 6 and 7. The capacity to produce epithelioma does not always run parallel with the capacity to produce sarcoma.

A preliminary study has been made of the occurrence of tumors of the lung and stomach in mice receiving these compounds. The derivatives of 3,4-benzphenanthrene, and of acridine and fluorene, appear to produce tumors of the lung more readily than do the compounds derived from 1,2-benzanthracene. Of the derivatives of 1,2-benzanthracene, the 5-n-butyl, 5-n-amyl, and 5-n-hexyl compounds appear to be most active in producing tumors of the lung, while they are deficient in power to produce sarcoma. None of the dibenzfluorenes tested produced sarcoma. Tumors of the stomach occurred most frequently in mice receiving acridines, and these compounds can produce sarcoma. The authors emphasize that much further study of these multiple tumors, and of tumors remote from the site of application, is necessary before any conclusion can be drawn as to their causation. For this investigation strains of mice are required in which the spontaneous incidence of all such tumors is known.—E. L. K.

BECK, S., and P. R. PEACOCK. (Research Dept., Glasgow Roy. Cancer Hosp., Glasgow, Scotland.) The latent carcinogenic action of 3:4-benzpyrene; results of intermittent application to the skin of mice. Brit. J. Exper. Path., 21:227-230. 1940.

Series of mice were painted on the skin with benzpyrene (0.3% in benzene) for varying periods and with varying frequency. Fluorescence tests indicated that 1 application of hydrocarbon persists for 4 days. Painting every 8th day (*i.e.* skin free from benzpyrene every alternate 4 days) for 6 months gave as many tumors as did painting biweekly for 3 months, although this 8th day painting lengthened the latent period. Biweekly painting gave in 1 month no tumors; in 2 consecutive months 6 tumors in 17 mice; in 3 consecutive months 18 tumors in 20 mice. Three series were then painted biweekly thus: 3 months' painting separated by intervals of 1 month; 3 months' painting separated by intervals of 2 months; 3 months' painting separated by intervals of 3 months. The yield of tumors was the same in each of the 3 series, 9 tumors in 19 mice, although the latent period lengthened *pari passu* with the separation period between the painting months.

The authors draw attention to the danger of working with hydrocarbons since contact with the skin initiates changes which are not reversed after the apparent disappearance of the hydrocarbon.—I. H.

BERGMANN, W. (Dept. of Chem., Yale Univ., New Haven, Conn.) Über vermutliche Beziehungen zwischen Cholesterin und cancerogenen Stoffen. The possible relation between cholesterol and carcinogenic compounds. Ztschr. f. Krebsforsch., 48:546-552. 1939.

Chemical and biological relationships of cholesterol to carcinogens are discussed. Since conversion of a 4-membered ring steroid to a penta-cyclic carcinogen requires 2 steps; (1) the formation of a new ring by cyclization of the steroid side chain, and (2) the dehydrogenation of the cyclohexane rings to aromatic rings, Bergmann suggests that it can be assumed that only those steroids with side chains long enough to form a new ring can serve for the biological formation of carcinogens. Experimental evidence for step (2) is discussed. The relation of substitution at positions 5,6,9, and 10 in the benzanthracenes to carcinogenic activity is discussed and it is pointed out that a compound, as yet unknown, worthy of investigation for carcinogenic activity is the 7,9 methyl cyclohexane derivative of methylcholanthrene, named "steranthrene" by Bergmann. Steranthrene is pictured as being readily formed from cholesterol by the 2 steps mentioned. It contains all the carbon atoms of cholesterol except the angular methyl group. Since it is a benzanthracene derivative with substitution at positions 5,6,9, and 10, the compound should prove to have high carcinogenic activity.

Roffo's theory of the conversion of cholesterol into carcinogenic substances by ultraviolet light is discussed. Unpublished results by Bergmann and Strong have shown that cholesterol irradiated with ultraviolet light possesses no carcinogenic properties in mice when applied in benzene solution to the skin, or injected in oil subcutaneously. The high incidence of neoplastic changes in the digestive tract of rats fed irradiated or cooked diets high in cholesterol, reported by Roffo, does not necessarily mean that cholesterol takes on carcinogenic properties after heat or light treatment.—P. P. C.

CHALMERS, J. G., and A. H. M. KIRBY. (Research Dept., Glasgow Roy. Cancer Hosp., Glasgow, Scotland.) The elimination of 3:4-benzpyrene from the animal body after subcutaneous injection. I. Unchanged benzpyrene. Biochem. J., 34: 1191-1195. 1940.

Data are given on the elimination of benzpyrene, partly unchanged and partly in the form of a fluorescent derivative, in the bile and excreta of the fowl and rat after intravenous or subcutaneous injection. The 2 compounds can be separated by alcohol and acetone extraction followed by chromatographic adsorption from benzene solutions on alumina. Tests where known amounts of benzpyrene were added showed that this method for assaying the unchanged hydrocarbon gave a recovery of 90%. Three rats were given 20 mgm. benzpyrene each in olive oil, subcutaneously, and the pooled excreta collected over 1 month when these no longer gave fluorescent extracts. It was found that 1% of the total benzpyrene injected had been eliminated unchanged, chiefly in the feces. Of the 99% to be accounted for, a large part was eliminated as the fluorescent derivative, and some was still encapsulated at the site of injection, as seen by the fluorescence of the tissues in ultraviolet light.—I. H.

COOK, J. W., C. L. HEWETT, E. L. KENNAWAY, and N. M. KENNAWAY. (The Chester Beatty Research Inst., The Roy. Cancer Hosp. (Free), London, England.) Effects Produced in the livers of mice by azonaphthalenes and related compounds. Am. J. Cancer, 40:62-77. 1940.

The azonaphthalenes studied in this paper are structurally related to certain dibenzcarbazoles and azo compounds known to be active in producing neoplastic changes in the livers of mice. The following compounds were administered to 444 mice, either by feeding, painting the skin, or by subcutaneous injection: 1,1′-azonaphthalene; 4-amino-1,2′-azonaphthalene; 1,2′-azonaphthalene; 2,2′-azonaphthalene, and 2,2′-diamino-1,1′dinaphthyl. It was found that 2,2′-azonaphthalene and its reduction product 2,2′-diamino-1,1′-dinaphthyl induce new growth in the liver either of a cholangiomatous or hepatomatous character. The other compounds showed little or no activity. Structurally corresponding azonaphthalenes and dibenzcarbazoles corresponded also in their ability or lack of ability to produce neoplastic changes in mouse livers. However, the ability of 3,4,5,6-dibenzcarbazole to produce sarcomas at the site of application was not reproduced by the corresponding azonaphthalene. Large members of other organic molecules were tested on mice for their effects on the liver. The only group being active was that containing derivatives of 3,4-benzphenanthrene. Finally, a group of water-soluble azo dyes used extensively in Great Britain for coloring food stuffs was tested, again on mouse livers, without positive result.—L. L. W.

DAVIS, W. W., M. E. KRAHL, and G. H. A. CLOWES. (Lilly Research Labs., Indianapolis, Ind.) Interactions between polycyclic hydrocarbons and sterols in mixed surface films at the air-water surface. J. Am. Chem. Soc., 62:3080-3098. 1940.

Preliminary observations on the interactions of carcinogenic and related hydrocarbons with various sterols in mixed surface films of the 2 classes of substances on water have been published (Am. J. Cancer, **36**:98-109. 1939; **37**:453-454. 1939). This paper reports a more intensive investigation of the properties of hydrocarbon-sterol mixed films with special reference to the role of these solutions and molecular associations in the biological effects of carcinogenic hydrocarbons. The apparatus and procedure for making force-area curves were the same as those previously described. A large number of sterols and hydrocarbons, including 1,2,5,6-dibenzanthracene and 20-methylcholanthrene, were studied. In some experiments a kymograph was used to make an automatic permanent record of the force-area relationships. The authors' summary follows:

"From measurements on surface films at the air-water interface, the molecular areas of 9 sterols have been determined. Other data regarding areas of sterols in surface films have been assembled and compared with previously published x-ray measurements on the dimensions of sterol molecules.

"P-A (pressure-area) and A-P (area-pressure) measurements have been made for a series of mixed films, each containing a sterol and a hydrocarbon in known ratio. Experiments of this type were performed at a number of hydrocarbonsterol ratios with each of 35 hydrocarbons (chiefly polycyclic compounds including or related to the carcinogenic hydrocarbons) in combination with cholestanol or with cholesterol. Certain representative hydrocarbons were also used with each of the following: *epi*-cholestanol, coprostanol, ergosterol, neoergosterol, sitosterol, and stearic acid.

"From analysis of data on mixed films obtained in this way it has been found that most of the hydrocarbons studied display sufficient reactivity toward sterol molecules to be held between the sterol ring systems at the air-water surface. The hydrocarbon molecules are apparently held in the mixed films in 2 principal ways: (a) In a 2 dimensional solution which the hydrocarbon molecules can enter or leave reversibly; the logarithm of the mole fraction of hydrocarbon held in such solution, at film pressures below 15 dynes, varies linearly with the pressure applied to the film by the movable barrier. At any given pressure the extent of this solubility depends on the structure of both the hydrocarbon and the sterol. (b) In association complexes with sterol molecules, 1 hydrocarbon molecule apparently is held between 2 appropriately oriented sterol molecules. For such association to occur the hydrocarbon must have a favorable molecular configuration with respect to ring sys-

tem and side chain. Any influence which favors a high degree of orientation among the solvent sterol molecules, such as low temperature or the presence of a third film component which leads to advantageous packing, favors this association. Any influence which disrupts the sterol orientation, such as high temperature, unfavorable sterol substituents or double bonds, or presence of an improperly packing third film component, detracts from the possibilities for formation of such hydrocarbon-sterol complexes.

"The molecular areas have been determined for most of the hydrocarbons studied and compared with the dimensions of the same hydrocarbon molecules in the crystal, as obtained from previously published x-ray data. Molecular areas for certain representative hydrocarbons have also been determined at temperatures from 8° to 40°.

"It is suggested that the solution type of interaction may possibly have some significance in the transport of polycyclic hydrocarbons in the animal organism. The association type of interaction, which is especially pronounced when the hydrocarbon-sterol films contain a fat, stearic acid, or a phosphatide as a 3rd component, may possibly provide a mechanism whereby the polycyclic hydrocarbons may be bound into, and act as a modifying influence in biological structures."

The paper contains 6 tables of quantitative data and 9 graphs.—S. B-J.

DES LIGNERIS, M. J. A. (The South African Inst. for Med. Research, Johannesburg.) Precancer and carcinogenesis. Am. J. Cancer, 40:1-46. 1940.

The author re-emphasizes the importance of studying the "stage of preparation" or precancerous state of tissues later developing true neoplasms. He feels that not only will such studies lead to the institution of earlier, and so more successful therapy, but also that they may throw light upon the mechanism of the neoplastic process itself. Following a brief review of the pertinent literature, he reports the following experimental studies:

Histological examination of tissues from mice and rats injected or painted with carcinogens (3,4-benzpyrene, methylcholanthrene) were made during the long latent period preceding cancer development. Although a progression of proliferative changes was noted in the tissues leading up to the neoplasms, morphological study of individual cells failed to reveal just when the neoplastic change took place. It was noted that after a certain period of specific irritation by carcinogens, tissues would go on to cancerization under the influence of nonspecific irritation (scalding). In contrast to the well recognized precancerous changes observed in the above studies, no such progression of events occurred in the tissues of fowl injected with the Rous sarcoma virus. The tumors appeared to arise from blood and tissue macrophages which showed sudden malignant transformation. No clearly defined precancerous state could be found in mice developing spontaneous mammary carcinomas.

In his concluding section the author states his belief that most cells show a lack of susceptibility towards spontaneous cancerization, but that they are made more and more susceptible to this change by the action of specific carcinogens throughout the precancerous period. Hence the need for recognition and eradication of this stage in the treatment of human malignant disease.—L. L. W.

DUNNING, W. F., M. R. CURTIS, and M. J. EISEN. (Dept. of Cancer Research, Columbia Univ., New York, N. Y.) The carcinogenic activity of methylcholanthrene in rats. Am. J. Cancer, 40:85-127. 1940.

Seven inbred and interbred strains of rats were injected with either 0.5 or 1.0% of methylcholanthrene in paraffin wax. Rats were injected in from 1 to 6 foci. It was found that a total dosage of 12 mgm. of methylcholanthrene divided in 6 foci was sufficiently toxic to cause death of a majority of the animals within 90 days. With this maximum dosage, no tumors were observed in less than 60 days. From data accumulated, it was further found that the animals' age at time of injection was not a factor in initiating the malignancy or in determining the length of its latent period. Although rats definitely underweight were more susceptible to the toxic action of the chemical, they did not seem more prone to develop induced tumors than those which were over the average weight.

The weaker solution of methylcholanthrene (0.5%) produced a larger proportion of tumors than the stronger (1.0%). By comparing a previous experiment with benzpyrene the data indicate that 0.5% methylcholanthrene is approximately equal in carcinogenic potency to 1.0% benzpyrene, and that 1.0% methylcholanthrene induces tumors in a shorter average time than similar doses of benzpyrene. The tumors induced by the 2 concentrations of methylcholanthrene were chiefly fibrosarcomas. The average diameter of those induced by 0.5% of the chemical was larger than that of those induced by the 1.0% solution after an equal growth period. No difference in the growth rates of tumors in the inbred and interbred strains was found. Hyperplasia of mammary duct epithelium and marked squamous metaplasia of these structures was often observed in the rats with tumors. Occasionally a squamous carcinoma or adenocarcinoma of the breast resulted.—L. L. W.

ENGELBRETH-HOLM, J. (Cancer Research Lab. of The Danish Anti-Cancer League, Copenhagen, Denmark.) Acceleration of the development of mammary carcinomas in mice by methylcholanthrene. Cancer Research, 1:109-112. 1941.

Twenty-seven females and 26 males of Little's dilute brown mice were painted once a week with 0.5% methylcholanthrene in benzene on various parts of the skin. All the 19 surviving females developed mammary carcinoma. Seventeen of the animals had more than 1 breast cancer. The tumors developed far earlier than is usual in this strain, the animals dying when from 8 to 10 months old as contrasted with the untreated mice which survived 13 to 15 months. No mammary carcinoma occurred in the males, but after the age of 10 or 12 months, there were some cases of papillomas and carcinomas of the skin. Methylcholanthrene thus accelerated the development of mammary carcinoma in the females.—Author's summary.

ENGELBRETH-HOLM, J., and H. LEFÈVRE. (Cancer Research Lab. of The Danish Anti-Cancer League, Copenhagen, Denmark.) Acceleration of the development of leukemias and mammary carcinomas in mice by 9,10-dimethyl-1,2-benzanthracene. Cancer Research, 1:102-108. 1941.

Reports are given of the results of experiments with 9,10-dimethyl-1,2-benzanthracene administered to 2 inbred strains of mice, a strain with high incidence of mammary carcinoma and low incidence of leukemia, Dlb, and a strain with high incidence of leukemia, Aka. Pronounced acceleration of the tumors characteristic for the strains, evident in increased incidence and especially in distinctly earlier appearance of the particular tumor, has been produced in these strains of mice by both subcutaneous injection of, and painting with 9,10-dimethyl-1,2-benzanthracene.—Authors' summary.

MORTON, J. J., and G. B. MIDER. (Dept. of Surg., Univ. of Rochester Sch. of Med. and Dentistry, Rochester, N. Y.) Some effects of carcinogenic agents on mice subject to spontaneous leukoses. Cancer Research, 1:95-98. 1941.

The appearance of leukosis in dilute brown mice may be hastened by painting them with: (a) a 0.5% solution of methylcholanthrene in benzene, (b) a 0.25% solution of methylcholanthrene in benzene, (c) a 0.25% solution of methylcholanthrene in acetone, or, (d) a 0.5% solution of 3,4-benzpyrene in benzene. Painting with benzene alone does not have

an effect of the same magnitude. The incidence of leukosis in line 212 of the dilute brown strain is greater after painting with methylcholanthrene than after painting with benzpyrene. Preliminary reports of the alterations in the blood leucocytes that precede the disease are presented.

Leukosis was found in old Buffalo, new Buffalo, and C3H mice after painting with a 0.5% solution of methylcholanthrene in benzene but not in the C57 black or C albino strains. All of these mice are known to develop spontaneous leukosis although the incidence is not established.—Authors' summary.

RILEY, J. F. (Scottish Emergency Hosp. Service.) Sulphydryl compounds and wound repair. Brit. M. J., 2:516-519. 1940.

In the course of a review of the cytological processes involved in wound healing, the author mentions that he carried out the following experiment. Mice (actual number not stated) were painted with one of the carcinogenic hydrocarbons for a period of time short of producing tumors. Wounds of a standard size were then made in the areas of depilation. One-third of the animals were kept as controls, ⅓ of the wounds were treated with fresh embryo extract, and the rest were dressed with heparin ("as used in delayed-culture methods *in vitro*"). "Although one or two tumours arose in the controls, by far the greater number appeared in the heparinized—*i.e.* inhibited—wounds, and none at all in those treated with embryo extract." This experiment is quoted in support of Haddow's view that inhibition of mitosis is responsible for the induction of tumors. The author therefore cautions against attempts to stimulate epithelization of wounds by inhibiting fibroblastic growth.—R. J. L.

ROFFO, A. H. (Inst. de med. exper. para el estud. y trat. d. cáncer, Buenos Aires.) La producción de carcinoma en el conejo por destilado de fuel-oil. Production of carcinomas in rabbits by distillates of fuel oil. Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, 54:385-396. 1940.

In preliminary notes (Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, **50**:77. 1939 and **52**:1. 1939) the author described the induction of lung tumors in rats made to breathe gases of combustion from fuel oil. In the present study 3 fractions were procured by distillation of fuel oil at the following temperatures (a) 220-240° C., (b) 270-300° C., and (c) 300-330° C. Each of these materials was tested for carcinogenic potency by daily painting of the ears of 10 rabbits. Fraction (a) although very toxic proved to be ineffective; fractions (b) and (c) proved to be highly effective, each inducing 80% of tumors. The first lesions were detected from 85 to 300 days after the beginning of treatment, and appeared earlier in rabbits of group (c). The different stages in the development of the cancers is well illustrated by 33 photographs of gross and microscopic preparations. Spectographic and fluorescence studies showed in the active fractions the same characteristics as those of carcinogenic hydrocarbons, and the same holds true for fractions secured from the exhaust of both ordinary and Diesel engines.—M. D-R.

Hormones

BUTENANDT, A. (Kaiser-Wilhelm-Inst. für Biochem., Berlin-Dahlem, Germany.) Fortschritte in der Biochemie der Steroide, insbesondere der Sexual-hormone. Progress in the biochemistry of the steroids, particularly the sex hormones. Veröffentl. d. Berliner Akad. f. ärzt. Fortbildung., 6:46-64. 1940.

This review article covers recent advances in the field of steroid hormones. The special topics discussed are the following: gonadal and adrenal hormones; chemical synthesis and enzymatic conversion; biochemical synthesis of testosterone; genetic relationships and excretion; steroid metabolism and pathology; and the relation of the steroids to carcinogenic compounds. The chemical relationships of the different steroids are illustrated by structural formulas. The relation of the steroids to carcinogenic compounds is discussed in detail. Butenandt refers to experiments now in progress in his laboratory in which the nonsaponifiable fraction of liver and bile from normal and cancer patients, and of tumors, was tested for carcinogenic activity in the mouse, by skin painting and subcutaneous injection. To date tumor and bile fractions have been tested for over 14 months, and the liver fraction for over 11 months. In contrast to the findings of Schabad no true carcinomas or sarcomas, similar to those seen after treatment with the carcinogens, have been observed at the treated sites. The noncarcinogenic activity of the estrogens is stressed and data from experiments still in progress are presented to support this view. A total of 2,500 mice of BlH strain, which has a high incidence of spontaneous breast tumors, has been observed for over 3 years. Starting at 3 weeks of age the animals, male and female, were treated weekly in doses of 30 to 100 γ of estrone, estrone benzoate, estradiol benzoate, and equilin benzoate. The estrogens were either injected subcutaneosuly in olive oil or painted on the skin in chloroform solution. A total of 550 breast tumors was diagnosed. In the females there was no significant difference in either the number or time of appearance of the breast tumors in the estrogen treated and untreated animals. However the animals treated with the benzoates of estrone, estradiol, and equilin produced breast tumors on the average 2 months earlier than the estrone treated and control females. Treatment of male animals in a similar manner led to a breast tumor incidence no higher than that of the untreated females. This merely means, according to Butenandt, that the males have been feminized by the estrogen therapy to the point where the hereditary influences in the strain can cause breast tumors.—P. P. C.

E. C. DODDS. The new oestrogens. Edinburgh M. J., 48: 1-13. 1941.

A review (Cameron Prize Lecture, University of Edinburgh) of the practical and theoretical significance of the synthetic estrogenic analogues, with special reference to the stilbestrol series.—A. H.

GEIST, S. H., and U. G. SALMON. (Mount Sinai Hosp., New York, N. Y.) Are estrogens carcinogenic in the human female? Am. J. Obst. & Gynec., 41:29-36, 1941.

Various estrogens were administered to women suffering from an estrogen deficiency. The hormones were given in individual doses ranging from 10,000 I.U. to 150,000 I.U. The total amount administered varied from 500,000 I.U. to 23,400,000 I.U. The vaginal mucosa resembled that of the adult cyclical female following the administration intramuscularly of 200,000 to 2,000,000 I.U. of estradiol benzoate. When a total of 20,200,000 units of the estrogen were administered, excessive or abnormal proliferation of the vaginal epithelium did not appear. The atrophic endometrium responded more slowly than the vaginal mucosa to estrogenic stimulation. Uterine bleeding usually occurred after the patient had received approximately 1,000,000 to 5,000,000 I.U. during 4 to 6 weeks. Biopsies performed during the period of bleeding showed cystic glands. Following bleeding the endometrium returned to the atrophic state. When the endometrium was stimulated continuously for from 6 to 38 months it did not show any greater proliferative activity than after a 4 to 6 week stimulation. The authors feel that within the limits of the dosage used (up to 53,400,000 I.U.) there appears no evidence to justify the fear that carcinoma of the genital tract may result from the therapeutic use of estrogens. It is felt that relatively huge doses administered over relatively long periods of time are necessary to produce carcinoma experimentally in rodents.—A. K.

GRUMBRECHT, P. (Univ. Frauenklinik, Freiburg, Germany.) Pathologische Auswirkungen des Follikelhormons. Pathological effects of estrogenic hormones. Archiv. f. Gynäk., 170:1-59. 1940.

Over 500 intact or ovariectomized female rats (120-125 gm.) received daily subcutaneous injections of 1000 I.U. (benzoate units) daily in 0.1 cc. of oil. A mixed diet was fed. Vaginal smears were taken at daily or weekly intervals. Animals were killed at intervals of 10 days, and the uterus, vagina, mammary glands, liver, spleen, bone marrow, gut, bladder, lymph nodes, kidney, and endocrine glands were studied histologically. Some rats were treated more than 1 year. The injected rats gained weight for 50 to 60 days and then stopped growing. The large amount of oil injected accumulated and abscesses formed in the subcutaneous tissue. Scattered areas, or generalized squamous metaplasia of the uterine epithelium appeared. Pyometra occurred frequently. The mammary glands were well developed and fibrous stroma increased but malignant growths did not appear. Hypophysial chromophobic adenomas appeared after prolonged treatment. The tumors contained little thyrotropic or gonadotropic hormone. The ovaries decreased to about ⅛ their normal size. Colloid storage increased in the thyroid and metabolism was reduced to 86% within 13 days. All blood cells decreased with continued treatment. The pathological changes are discussed from their sequence of appearance. A section is devoted to a discussion of the similarity of certain pathological states in man. The paper is illustrated with 37 photomicrographs or charts and 5 tables; 79 references are cited.—W. U. G.

MOHS, F. E. (McArdle Memorial Lab., Med. Sch., Univ. of Wisconsin, Madison, Wis.) The transformation of rat mammary adenofibroma to fibroma by androgens. Cancer Research, 1:151-153. 1941.

Androgenic hormone tends to transform mammary adenofibroma to pure fibroma in rats. After 25 or more weeks of exposure to physiologic amounts of androgens fibromatous transformation of adenofibromas occurred in 28 of 47 rats (59.6%) and partial transformation took place in the rest. Less than 25 weeks' exposure produced relatively little change in tumor histology. Adenofibroma was never transformed to fibroma in females or in estrogen-injected castrates, and only rarely so in untreated castrates. The fibromatous transformation produced by androgens does not depend upon inhibition of the growth of the tumor as a whole. Different strains of adenofibroma vary in the degree to which they respond to the fibroma-producing influence of androgens. No adequate explanation based on normal mammary physiology was found for the transformation of adenofibroma to fibroma. Only when the mammary epithelium is a part of a benign mammary tumor does it become sensitive to the inhibitory effect of androgenic hormone.—Author's summary.

WOOLLEY, G., E. FEKETE, and C. C. LITTLE. (Roscoe B. Jackson Memorial Lab., Bar Harbor, Maine.) Effects of castration in the dilute brown strain of mice. Endrocrinology, 28:341-343. 1940.

In male mice of the Jackson Laboratory dilute brown strain castrated at birth, or at 1 month or 6 months of age, hyperplasia of the adrenal cortex and in many cases growth of the mammary glands occurred. The changes in the adrenal preceded the mammary gland development and only feminizing effects were observed. Forty males were castrated at birth and 60 at later ages. Thirteen of these castrated at birth were examined when more than 300 days of age. All showed nodular hyperplasia of the adrenals consisting of nonpigmented cells, increasing with the age of the animal. The yellow-brown pigment characteristic of adrenal hyperplasia in older ovariectomized females was almost absent from males of comparable ages, indicating a less advanced hyperplastic condition. Six animals (averaging 644.2 days) showed extensive mammary gland growth (subbranching and alveoli) not found under 437 days. One male killed at 589 days of age had 2 mammary tumors. Similar adrenal changes and mammary gland growth occurred in males castrated at later ages. The hypophyses contained no castration cells but were enlarged and congested.—C. A. P.

Viruses

BALLS, A. K., E. F. JANSEN, and B. AXELROD. (Food Research Division, Bureau of Agric., Chem., and Engineering, U. S. Dept. of Agric.) A chemical study of the virus of infectious myxomatosis. Enzymologia, 8:267-272. 1940.

A dialyzable stabilizing factor for myxoma virus is present in myxoma tissue as well as in normal rabbit muscle and skin and in commercial beef extract. By keeping the virus in contact with a saline solution of the stabilizing factor, it has been possible to concentrate the virus 100 times over that of the original extract by sedimentation in the ultracentrifuge (34,000 *g* for 2 hours). No sediment was obtained when an extract of normal rabbit muscle was treated in a similar fashion. The suspended particles under the microscope resembled the elementary bodies of vaccinia virus. On a nitrogen basis, 9.4×10^{-12} gm. constituted a fatal dose. About ⅓ of the sediment consisted of a lipoid foreign to normal rabbit muscle. It is thought that the lipoid consists of a base bearing nitrogen and phosphorus and a steroid that is not cholesterol but possesses some acidic properties.—J. L. M.

CAMPBELL, J. A. (Nat. Inst. for Med. Research, London, England.) Influenza virus and the incidence of primary lung tumours in mice. Lancet, 2:486. 1940.

Mice, aged about 2 months, were vaccinated by peritoneal injections of virus vaccine which produced no symptoms. This was followed by intranasal instillation of live virus. The survivors, about 3 months old, were exposed for about 10 minutes once an hour to a cloud of dust; in all, 6 times a day on 5 days every week over a total period of a year. Of 23 mice thus treated only 13 survived 10 months or longer. Only 2 of these, aged 605 and 877 days, developed malignant tumors of the lungs; *i.e.*, 15.4%. The average incidence of lung cancer in 593 control mice, and 727 dusted mice of similar ages was 11.3% and 31.5%, respectively. Thus the influenza virus had, if anything, an inhibitory effect on the development of lung carcinoma.—R. J. L.

LEA, D. E. (Strangeways Research Lab., Cambridge, England.) Determination of the sizes of viruses and genes by radiation methods. Nature, London, 146:137-138. 1940.

This article reviews work in which radiation experiments have been used for determining the particle size of viruses and genes, and indicates possible extensions of the method. The inactivation of viruses and phages by ionizing radiations appears to be a simple process, in which a single ionization or cluster of ionizations is responsible for the inactivation of 1 particle. The lines of evidence leading to this conclusion are given, and a simple formula is elaborated connecting the inactivation dose (*ions per cm.*^{3}) with the particle volume. Examples of the method are given for phage 16 and the tobacco necrosis virus, the results being in good agreement with the sizes estimated from ultracentrifugation and ultrafiltration experiments. The production of sex-linked recessive lethal mutations in *Drosophila* by irradiation can be used to calculate the diameter of the gene. In this case a modification is introduced by which the ratio of the doses of 2 different radiations required to produce the same percentage of lethals is estimated. The experimental results with x-rays, neutrons, and α-rays are in agreement with the assumption that the x-chromosome contains 1,860 genes of average diameter 3.78 mμ. Other biologi-

cal actions of radiation which have been interpreted in terms of regions of special sensitivity in the organism are discussed, and calculations are given to justify the assumption that the lethal effect of radiations on bacteria can best be interpreted as the production of lethal mutation.—H. G. C.

Genetics

BARNES, W. A. (Dept. of Path., Cornell Univ. Med. Coll., New York, N. Y.) and R. K. COLE. (Dept. of Poultry Husbandry, Cornell Univ., Ithaca, N. Y.) The effect of nursing on the incidence of spontaneous leukemia and tumors in mice. Cancer Research, 1:99-101. 1941.

This study is based on over 600 fostered mice of the Ak, Rf, and Af strains observed from birth until natural death. The incidence of leukemia is low in stocks Rf and Af, and high in stock Ak. Mammary carcinoma is common in both virgin and bred mice of stock Af, while in stock Rf lung tumors are numerous. Mice of the stock studied having a low incidence of leukemia were not rendered leukemic by nursing with mice of a stock (Ak) having a high incidence of leukemia. Mice of a highly leukemic stock (Ak) fostered by mice of relatively nonleukemic stocks (Rf and Af) developed leukemia in lower percentage than their nonfostered relatives. Mice of stock Af having a high incidence of breast tumors failed to develop tumors, with a single exception, when nursed by mice of stocks having a very few breast tumors. Breast tumors did not develop in mice of these stocks with a low incidence of breast tumors when nursed by Af mice. The incidence of lung tumors was not significantly altered by fostering.—S. B-J.

BITTNER, J. J. (Roscoe B. Jackson Memorial Lab., Bar Harbor, Maine.) Changes in the incidence of mammary carcinoma in mice of the A stock. Cancer Research, 1:113-114. 1941.

Mammary carcinoma in strains of mice having a high incidence of this tumor may be reduced to a minimum by foster nursing. A line of mice with a low incidence of mammary carcinoma, but susceptible to the development of mammary carcinoma, may, without fostering by or association with mice of a strain with high incidence, acquire an active milk influence producing mammary carcinoma. Thus the line may change, in any generation, to a strain with high incidence of mammary carcinoma. It is possible, but has not been demonstrated, for mice of a strain with low incidence of mammary carcinoma, nonsusceptible to development of breast cancer, to acquire, without fostering, an active milk influence and still not develop mammary carcinoma. The active milk influence may arise *de novo* or by changes in the inactive milk influence. The most consistent explanation for these observations and the data on foster nursing is that the active milk influence is an agent or virus.—Author's summary.

BITTNER, J. J. (Roscoe B. Jackson Memorial Lab., Bar Harbor, Maine.) The variability of incidence of mammary carcinoma in inbred strains of mice. Cancer Research, 1: 115-120. 1941.

Observations on inbred strains of mice and on single sublines have shown that variation occurs both in the incidence of spontaneous mammary carcinoma and in the average age at which this tumor of the breast occurs. This indicates that inbred strains of mice or sublines of the same inbred stock are subject to mutational changes. In investigations involving comparisons of inbred strains and sublines, the mice to be used as controls should be bred and reared under the same conditions as the experimental animals.—Author's summary.

BURROWS, H. (The Chester Beatty Research Inst., The Roy. Cancer Hosp. (Free), London, England.) Decline in the incidence of mammary cancer in mice of an inbred strain. Cancer Research, 1:121-122. 1941.

Considerable variation in the incidence of mammary carcinoma developed in several lines of Little's Dba strain of mice maintained since 1935 by inbreeding through brother-sister matings at this laboratory in London. All offspring were nursed by their own mothers. The percentage incidence of mammary carcinoma in 5 lines, A,B,C,D, and E, was respectively 63.1, 57.5, 57.4, 56.5, and 10.1. The average age at which tumors developed in the E line mice was no greater than that at which they developed in the A, B, C, and D lines. No cause of the divergence of the E mice from the biological behavior of the rest of the Dba stock has yet been found.—S. B-J.

MURRAY, W. S. (N. Y. State Inst. for Study of Malignant Diseases, Buffalo, N. Y.) Studies on the inheritance of mammary carcinoma in the mouse. Concentration of the extrachromosomal factor. Physiological stability of the individual. Cancer Research, 1:123-129. 1941.

Investigations attempting to put the occurrence of spontaneous mammary carcinoma in mice upon a genetic basis have led to controversy. The author discusses the difficulties and records observations on 3 stocks of mice. His conclusions are:

"An influence which is transmitted by the mother but not carried in the chromosomes is instrumental in causing her young to have mammary carcinoma. The inbred stocks used apparently transmit this influence in degrees or concentrations which are characteristic of each stock. Physiological stability, as measured by the length of life or ability to survive, is apparently transmitted through the chromatin. The amount of spontaneous mammary cancer in hybrids of these 3 stocks seems to be dependent upon 2 factors: (a) the concentration of the extrachromosomal influence transmitted by the mother; (b) the physiological resistance of mice of various genetic constitutions to this stimulus."

The paper includes 2 tables and 7 graphs.—S. B-J.

PYBUS, F. C., and E. W. MILLER. (J. H. Burn Research Lab., Roy. Victoria Infirmary, Newcastle upon Tyne.) The gross pathology of spontaneous bone tumours in mice. Am. J. Cancer, 40:47-53. 1940.

The authors report on the bone tumors found in 300 mice of the Simpson strain. Seventy-four % of the animals with tumors were females. The mean age for tumor development was 16.6 months for females and 18.1 months for males. Tumors involving multiple bones were very common. Isolated tumors involving 1 bone were found most frequently in the spine, femur, tibia, and jaw. Metastases were frequent, appearing in the lungs, liver, kidneys, spleen, and peritoneum most often. The lesions varied from the earliest neoplastic changes, visible in the bones as an alteration of color (due to an abnormal proliferation of osteoblasts), to diffuse cancellous enlargement, or circumscribed osteoma, or spindle cell sarcoma, and cartilagenous tumors. Excellent photographs of the tumors are included.—L. L. W.

PYBUS, F. C., and E. W. MILLER. (J. H. Burn Research Lab., Roy. Victoria Infirmary, Newcastle upon Tyne.) The histology of spontaneous bone tumours in mice. Am. J. Cancer, 40:54-61. 1940.

The histology of the various types of spontaneous bone tumors found in the Simpson strain of mice is described. The main classes of tumors are solid and cancellous osteoma, osteosarcoma with or without giant cells, and chondro-osteosarcoma. Tumors in which bone and osteoid tissue predominate are the most frequent. Photomicrographs of the various types of tumors are included.—L. L. W.

Physical Factors

FULLER, R. H., E. BROWN, and C. A. MILLS. (Lab. for Exper. Med. and Dept. of Path., Coll. of Med., Univ. of Cin-

cinnati, Cincinnati, O.) Environmental temperatures and spontaneous tumors in mice. Cancer Research, 1:130-133. 1941.

Virgin female Dba mice kept under conditions of moist warmth (90-91° F. and 60-70% relative humidity) showed a striking decrease in spontaneous tumor incidence. Tumors that did appear under these conditions grew more slowly and took over twice as long to kill their hosts as tumors in animals kept at cooler, more stimulating temperatures (65° F.). Tumor incidence at 70-75° F. was practically the same as at 65° F., but the masses grew more slowly. The time interval from discovery of the tumor to the death of the animal was 28 days at 65° F., 47 days at 70-75° F., and ± 60 days at 90-91° F. That the lowered tumor incidence observed in warm environments represents a real suppression rather than merely a delay to a later age is indicated by a comparison of human cancer death rates by age groups in northern and southern states. Northern rates rise much more rapidly with advancing age than do those in the South. From these findings it would seem that cancer should be classed as a metabolic disease, closely linked to tissue combustion level, as are diabetes, pernicious anemia, hyperthyroidism, and Addison's disease.—Authors' summary.

Radiation

LASNITZKI, I. (The Strangeways Research Lab., Cambridge, England.) The effect of x-rays on cells cultivated in vitro. Brit. J. Radiol., 13:279-284. 1940.

Tissue cultures (from choroid and sclerotic of chick embryo) were exposed to a standard dose of x-rays (96.7 r) and the resultant changes studied over a period of 24 hours. The radiation caused a decline in mitosis, followed by an attempt at recovery. This was accompanied by a degenerative process due mainly to the breakdown of cells which had completed prophase. Since the results closely resembled those obtained in experiments *in vivo*, it is concluded that the effects of radiation in these dosages is independent of any effect on blood circulation.—A. H.

MITCHELL, J. S. (Dept. of Med., Univ. of Cambridge, Cambridge, England.) Increase in ultra-violet absorption of cytoplasm after therapeutic x- and gamma-irradiation. Nature, London, 146:272-273. 1940.

For this research a modification of Caspersson's ultraviolet microphotographic technic was employed, which permitted of a quantitative estimation of nucleic acid, or other known absorbing compounds in sections of tissues. Biopsy specimens were examined from cancer cases before and 80 minutes after x- and gamma-irradiation. There was found a significant increase in ultraviolet absorption of the cytoplasm after treatment. This was detectable after small doses of irradiation, such as 248 and 388 r but of 6 specimens examined after relatively high dosages (0.91-29.7 r/min.) there was increased cytoplasmic absorption. The magnitude of the absorption is consistent with the possibility that purine and pyrimidine derivatives may be responsible. Microchemical tests have demonstrated in some cases an accompanying positive pentose reaction in the cytoplasm. The absorbing substances can be removed from the cytoplasm in fixed tissues by acid hydrolysis followed by alcohol extraction. Clinically there was no obvious correlation between the response to treatment and the absorption change reported.—R. J. L.

RUSS, S., and G. M. SCOTT. (Barnato Joel Lab., The Middlesex Hosp., London, England.) The effect of x-rays upon tumour growth when the tumour itself is not irradiated. Brit. J. Radiol., 13:267-272. 1940.

When a dose of x-rays approximating 1,200 r was delivered to the tumor-bed of the Jensen rat sarcoma, the rate of growth of the tumor was considerably slowed and 30 of 82 tumors regressed completely. The number of untreated control tumors which disappeared spontaneously was 13 out of 190.—A. H.

SEABORG, G. T. (Dept. of Chem., Univ. of California, Berkeley, Calif.) Artificial radioactivity. Chem. Rev., 27: 199-285. 1940.

Since the discovery of artificial radioactivity by Curie and Joliot in 1934 its production and use in chemistry, physics, biology, and medicine has increased enormously. This review of advances is written from the point of view of the chemist and physicist. It is divided into the following sections: I. Introduction; II. General considerations; III. Methods of chemical identification and concentration; IV. Methods for detection of radiations; V. Types of reactions and methods of production; VI. Table of artificial radioelements (a complete list up to July 15, 1940, giving type of radiation, half-life, energy of radiation, and nuclear reactions by which the radioactive isotopes are formed); VII. Applications to chemistry. The review includes an extensive bibliography.—S. B-J.

Biochemistry and Nutrition—Chemotherapy

CASEY, A. E. (Dept. of Path. and Bact. of the Sch. of Med. of Louisiana State Univ., New Orleans, La.) Experiments with a material from the Brown-Pearce tumor. Cancer Research, 1:134-135. 1941.

From 1929 to 1939, 1,500 rabbits and 800 mice were treated with single injections of 3 cc. of an emulsion containing from 0.0001 to 0.1 gm. of the Brown-Pearce tumor. Transplantation of the tumor as much as 2 weeks before or 2 weeks after these injections resulted in increased metastases, shorter life, and greater mortality. The so-called "xyz" factor responsible for this increased malignancy can be filtered "through a Berkefeld V candle, withstands drying, is thermolabile (56° C.), can be preserved for some months, and differs from the spreading factor of Duran-Reynals." This material is the only one reported which "exerts a measurable specific and homologous stimulation of the growth and spread of the same mammalian tumor *in vivo* but has no such effect on any other tumor thus far tested." Nor has any other tumor material shown a similar effect on the Brown-Pearce carcinoma.—J. S. L.

DICKENS, F. (London, England.) The citric acid content of animal tissues. Chem. & Indust., 59:135. 1940.

Colorimetric determination by the pentabromacetone method showed that citric acid content of transplanted tumors was 3 to 4 times higher than that of normal organs. Values for citric acid in mgm. per 100 gm. of fresh tissue were: Walker-Guérin rat tumors, 16.6; Brown-Pearce rabbit carcinoma, 13.6; Crocker mouse sarcoma, 14.2. Like tumor tissue, whole embryos and newborn mice have high citric acid contents (12.4, 16.2 mgm. %), but so has the adult mouse (14 mgm. %) because of the relatively large amount of citric acid in the bones. A Crocker sarcoma about equal to the rest of the mouse in size contained 45% of the citric acid. Values for normal organs are given.—S. B-J.

GARAI, F. (Dept. of Path., Manhattan Eye, Ear, and Throat Hosp., New York, N. Y.) Heptyl aldehyde-sodium bisulfite: toxicity and effect on spontaneous mammary carcinoma in mice. Cancer Research, 1:144-145. 1941.

Tumor-bearing mice of C3H, Dba, and Paris R3 strains were injected with heptyl aldehyde-sodium bisulfite intraperitoneally and subcutaneously. The compound had a destructive effect on the kidneys and liver which out-weighed its partial retardation of the growth of spontaneous tumors.—J. S. L.

HESS, M. (Dept. of Path., British Postgraduate Med. Sch., London, England.) The localisation of acid azo dyes in tumours. J. Path & Bact., 51:309-311. 1940.

The tumors employed were the transplantable mouse carcinoma 63 of the Imperial Cancer Research Fund and the

Guérin rat carcinoma. Animals bearing these tumors were injected intravenously with aqueous solutions of Chicago blue 6B. The results confirm those reported by Ludford and Duran-Reynals in demonstrating that no dyestuff was segregated by the malignant cells. In addition to phagocytic cells there are always present, in tumors, degenerating cells which constitute an important factor in the localization of acid vital dyes.—R. J. L.

MATHERS, R. G. (Shrubsall Biochem. Labs., Westminster Hosp. Med. Sch., Univ. of London, London, England.) Glutamic acid in tumor hydrolysates. Cancer Research, 1:146-147. 1941.

Analyses for d(-) form of glutamic acid were made on the hydrochloric acid hydrolysates of 7 malignant tumors from human beings, normal ox heart, and normal calf liver. The amount of d(-) glutamic acid found in tumor tissue was small, 0.5 to 0.91% in yields of 55 to 81 mgm. of total glutamic acid. Only a slightly smaller amount, 0.33 to 0.48%, was found in normal tissue. The observations of Kögl and Erxleben were not confirmed.—S. B-J.

McEWEN, H. D., and F. L. HAVEN. (Dept. of Biochem. and Pharmacol., Sch. of Med. and Dentistry, Univ. of Rochester, Rochester, N. Y.) The effect of carcinosarcoma 256 on the water content of the liver. Cancer Research, 1:148-150. 1941.

The percentage of water in the livers of rats (young males, Wistar strain) bearing carcinosarcoma 256 was found to be significantly higher than that in the livers of normal rats. This increase in liver water occurred independently of the diets used and is not accounted for by a decrease in dry residue.—Authors' summary.

ROFFO, A. H. (Inst. de med. exper. para el estud. y trat. d. cáncer, Buenos Aires.) Existencia en la sangre y en la orina de cancerosos y embarazadas de un principio activo de crecimiento esplénico. Presence of a principle stimulating the growth of the spleen in the blood and urine of cancerous individuals and during pregnancy. Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, 54:415-451. 1940.

After reviewing his numerous papers on the subject since 1929, the author records in 33 tables the results obtained when rats are given a single injection of 0.5 cc. of blood or urine from pregnant women or women with cancer of the uterus. Several hundred animals were used. These injections cause hypertrophy of the spleen, with enlargement to 2 or 3 times its normal size. The effect becomes apparent 24 hours after the injection, attains its maximum between the 4th and the 10th day, and lasts for 900 hours. The active principle seems to be linked with the lipoid fraction. Normal blood and urine are devoid of any effect on the spleen. In the opinion of the author the increase in tissue cholesterol such as is present in processes of growth is responsible for this effect.—M. D-R.

RONDONI, P. (Universitätsinst. für exper. Path. und Krebsinst. zu Mailand.) Untersuchung über Tumorproteine: N- und P-Fraktionierungsversuche. Investigations on tumor proteins: N- and P-containing fractions. Ztschr. f. Physiol. Chem., 265:102-112. 1940.

A comparison of the protein fractions of tumor and normal tissues is dealt with. Employing the belief that one should compare a tumor only with the tissue from which it arose, the author has compared a benzpyrene sarcoma of the rat with the normal cutaneous and subcutaneous tissue of the same animal. The tissues were extracted with a 10% NaCl solution, and then the residues were further extracted with a 0.25% NaOH solution. From 50 to 60% of the total tissue protein is dissolved in this manner. The partition of N and P was then determined in the soluble fractions. Seventeen sarcomas and 7 cutaneous-subcutaneous tissue controls were examined.

The protein in tumor appears to be less firmly bound to the insoluble material of the cell than is that of the normal tissue. However the values are greatly scattered, and overlap considerably. Whereas approximately equal amounts of P are extracted with saline from tumor and normal tissues, about 40 to 80% more P is extracted from the tumor in the subsequent alkali treatment. The ratio of P to N in saline extracts is of the same order for normal cutaneous-subcutaneous tissue (0.124) and for the sarcomas (0.112), but in alkaline extracts it is significantly higher for the tumor tissue (0.125 as compared to 0.093 for the normal tissue). The author contends from these results that sarcoma contains a higher content of P-containing alkali soluble proteins than cutaneous-subcutaneous tissue.—J. L. M.

ROSS, M., and R. I. DORFMAN. (Lab. of Physiol. Chem., Yale Univ. Sch. of Med., New Haven, Conn.) An abortifacient substance in human urines. Cancer Research, 1:158-163. 1941.

Protein-like substances were concentrated from the urines of 6 men and 8 women by precipitation with 10% sodium tungstate, solution of the precipitate in a mixture containing 60% acetone and 4% ammonium hydroxide, and precipitation of the desired fraction from this solution by increasing the concentration of the acetone to about 80% and adding a few drops of acetic acid. These concentrates were tested for abortifacient action by subcutaneous injection in pregnant albino mice.

The urine concentrates of each of the 7 patients with cancer exerted some abortifacient action. Concentrates of urine from 3 normal men gave no abortifacient action. In the urine of 1 normal man there was some evidence of the abortifacient. The urine of all 3 of the noncancerous but otherwise ill women showed as much evidence of abortifacient material as did that of the cancer patients.

Egg albumin and prolactin had no abortifacient action. Peptone, injected in doses of 8 to 10 mgm. caused resorption and expulsion of fetuses. Gonatropic substances in doses ranging from 3.75 to 25.0 rat units per day for 3 days tended to produce death of fetuses and their retention in the uterus, but differed considerably from the abortifacient action of the extracts of urine.—S. B-J.

Von EULER, H., and B. SKARZYNSKI. (Biochem. Inst. der Univ. Stockholm, Stockholm, Sweden.) Über die Spaltung von Dipeptiden in carcinomatösen und nicht carcinomatösen Seren. On the splitting of dipeptides in cancerous and noncancerous serums. Ztschr. f. Physiol. Chem., 265:133-146. 1940.

The serums of cancerous and noncancerous patients and of dogs with Brown-Pearce tumors were found to contain small amounts of d-dipeptidase. d-Leucyl-glycine served as the substrate. The enzymatic reaction had to run for exceedingly long periods of time (6 days) in order to demonstrate even this small activity.—J. L. M.

Transplantation

BUNTING, H., L. L. WATERS, and H. W. RYDER. (Yale Univ. Sch. of Med., New Haven, Conn.) Studies on the incidence and development of tumor 15091a in susceptible and resistant strains of mice. Yale J. Biol. & Med., 13:387-396. 1941.

The growth of tumor 15091a was studied in pure strain mice in correlation with the histology. The tumor grew well in the ABC ALB and ABC-AC strains, but the Dba strain was relatively resistant, and no takes at all were observed in C57 BLK mice. Individuals of the Dba strain in which the tumor failed to grow or regressed were resistant to reinoculation. In the susceptible animals the tumor became necrotic at the center but grew at the periphery. Vascularization became evident on the 4th day. Failure of the tumor to grow was associated with the development of a vascular fibrous capsule accompanied by mononuclear phagocytes about the pyknotic tumor cells. Ultimately a scar resulted. The more slowly growing tumors in the

susceptible strains also possessed thick fibrous capsules. There was no evidence that the capsule in itself is responsible for failure of the tumor to grow.—A. A. L.

HALL, B. V. (Univ. of Illinois, Urbana, Ill.) Influence of the estrus cycle and the decidual reaction on transplanted intra-uterine tumors in mice. Yale J. Biol. & Med., 13:333-349, 1941.

Intra-uterine implantation of a rapidly growing sarcoma into Swiss, Longacre, and AKA mice was successful in 75% of cases, 20% less than upon subcutaneous inoculation. The number of takes was least if implantation was made at estrus (59%), and best at proestrus (82%), or in 4 to 6 day pregnant, pseudopregnant, or lactating animals (79%). Growth was poorest at estrus and metestrus and best at diestrus and in pseudopregnant animals. It may be significant that the uterine fluid is less alkaline as decidual reaction develops, than at estrus or metestrus. Histological studies of implantation and growth of the tumor implants showed that even minute injuries with the trocar are sufficient to permit the implantation of the sarcoma cells. Tumor masses in the lumen rapidly underwent necrosis. With the development of the decidual reaction, sarcoma cells rapidly invaded the entire wall of the uterus. Both estrus and copulation were observed in mice with large uterine tumors. Decidua induced by placing tumor masses in sensitized uteri developed and differentiated much like those induced by embryos.

Invasion of lymph channels resulted in metastases in the lumbar lymph glands within 5 days after implantation of the tumor in the uterus.—A. A. L.

HOBBS, J. E., and A. R. BORTNICK. (Dept. Obst. and Gynec., Washington Univ. Sch. of Med., St. Louis, Mo.) Endometriosis of the lungs. Am. J. Obst. & Gynec., 40:832-841, 1940.

Endometrium of large rabbits previously treated with estrone was minced in saline and injected into the ear veins of the donors. Eight of 12 rabbits had endometrial tissue in their lungs 1 to 3 months later. Some of the pulmonary implants obliterated vessels and penetrated the walls of blood vessels. Thirteen other rabbits similarly received endometrial tissue and were mated or injected with stilbestrol. The endometrial tissue in the lungs showed decided changes in the pregnant rabbits and responded characteristically in rabbits receiving stilbestrol. The observations were discussed from the standpoint of vicarious menstruation and origin of benign and malignant tumors in women. The paper is illustrated with 11 photomicrographs.—W. U. G.

Tissue Culture

DOLJANSKI, L., and M. PIKOVSKI. (Dept. of Exper. Path., Hebrew Univ. Jerusalem.) Cultivation of the agent of fowl leukosis in vitro. Nature, London, 146:302-303, 1940.

The virus cultivated was the hemocytoblastosis strain Tl of Engelbreth-Holm; the technic employed the Carrel flask method. Two types of experiments were carried out. Bone marrow and fragments of heart muscle were cultivated separately in the plasma of leukotic fowls, and also colonies of fibroblasts were bathed for 1 hour in cell-free ultrafiltrates of heart and spleen extracts of leukotic fowls, and then cultivated in a mixture of 1 part of normal plasma to 2 parts of the ultrafiltrate. Both types of cultures were washed at intervals and re-fed with normal plasma (method of Parker and Fischer). Growth of virus infected cultures has been maintained for 86 days. Inoculation into fowls of ultrafiltrates of the fluid used for washing these cultures, and of the cultures themselves, gave positive results. It is thus demonstrated that cultivation of the virus of fowl leukosis *in vitro* does not necessitate the presence of primitive blood cells, since it can be cultivated in the presence of fibroblasts.—R. J. L.

FAVORITE, G. O., and F. S. CHEEVER. (Dept. of Bacteriol. and Immunol., Harvard Univ. Med. Sch., Boston, Mass.) Observations on the Brown-Pearce carcinoma in roller tube tissue cultures. Cancer Research, 1:136-143, 1941.

The roller tube culture technic appeared to furnish a practicable method for the maintenance *in vitro* of the specific cells of the Brown-Pearce carcinoma. After 44 days in tissue culture the carcinoma cells still retained their ability to produce malignant growths in susceptible animals. Definite evidence that these carcinoma cells proliferated *in vitro* was not obtained. The round tumor cells retained their characteristic morphology throughout the course of the experiments and no transition to a spindle-shaped fibroblast was observed. Serums and tissue extracts from animals immunized against the tumor showed no definite capacity to exert a cytotoxic action on the tumor cells *in vitro*.—Authors' summary.

JACOBY, F. (Dept. of Physiol. and Cancer Lab., Med. Sch., Birmingham, England.) Architecture of colonies of a pure strain of fibroblastic sarcomatous cells derived from a dibenzanthracene mouse tumour. Nature, London, 146:301-302, 1940.

A pure strain of malignant cells derived from a spindle cell sarcoma induced in a mouse by 1,2,5,6-dibenzanthracene has been grown in flasks on a fowl plasma coagulum and fed with either heparinized fowl plasma, or a mixture of fowl serum and a little chick embryo extract. The cells tended to grow in close association with one another forming branching ribbon-like strands or broad sheets, which resembled the epithelial type of growth *in vitro*. It is suggested that this type of growth results from the inability of the sarcoma cells to liquefy the heterologous plasma in the absence of chick embryo extract, or when it is present in a low concentration. This pure strain of sarcoma cells has been cultivated *in vitro* for 215 days. After 134 and 181 days *in vitro* cultures were inoculated into mice, and although they had been grown in completely heterologous media, they gave rise to typical spindle celled sarcomas.—R. J. L.

PEACOCK, P. R., and R. I. SHUKOFF. (Research Dept., Glasgow Roy. Cancer Hosp., Glasgow, Scotland.) Use of desiccated chick embryo in tissue culture technique. Nature, London, 146:30-31, 1940.

In modifying the technic of Borger and Zenker, who employed dried chick embryo extract, the authors have shown that a desiccate of chick embryo is equally efficacious. For preparing cultures they add an equal volume of distilled water to their desiccate. With this medium they have grown normal and malignant tissues—chick fibroblasts for 4 weeks, the Jensen rat sarcoma for 5 weeks, the Fujinami fowl sarcoma for 4 to 6 weeks, and a nonfilterable fowl sarcoma (GRCH/15) for 4 to 6 weeks.—R. J. L.

General Reviews

BUTENANDT, A. (Kaiser-Wilhelm-Inst. für Biochem., Berlin-Dahlem, Germany.) Neuere Beiträge der biologischen Chemie zum Krebsproblem. Recent contributions of biochemistry to the cancer problem. Angew. chem., 53:345-368, 1940.

This is a comprehensive review of the biochemical developments in cancer research discussed under the following headings: A viruses and tumor-producing agents; B carcinogenic compounds; C steroids and steroid hormones and their relation to the cancer problem; D biological relation between carcinogenic compounds and viruses—mode of action of cancer-producing compounds; E chemistry of tumors.

Under A the work on the Rous chicken sarcoma, Shope rabbit papilloma, and rabbit myxomatosis, and their respective viruses, is discussed. The viruses are considered as a group of carcinogenic compounds characterized by their high molecular weight and protein character. The discussion under B deals

with the various types of carcinogenic compounds of known chemical structure, and includes the aromatic hydrocarbons, the azo dyes, and the quinoline dye, styryl 430. The chemical and carcinogenic properties of those compounds are discussed. Section C on the steroids is taken up in more detail. Experiments in collaboration with Dannenberg and Friedrich-Freska which have been going on for 3 years are quoted as failing to support the claims of Schabad. Pathological changes are noted following injection into mice of the nonsaponifiable fraction from livers of cancerous patients, but no typical epitheliomas or sarcomas, such as are produced by the known steroid carcinogens, have been observed. Butenandt is emphatic in stating that the naturally occurring follicular hormones and their esters cannot be considered as carcinogenic in the same sense as the known carcinogenic hydrocarbons. Since the so-called carcinogenic action of the estrogens is seen only in males or castrated females of mice strains with a high incidence of spontaneous mammary carcinoma, the effect of the estrogens is that of bringing about proliferation of the mammary gland, which permits the factors responsible for the high spontaneous incidence to operate. The fact that stilbestrol and related compounds act in a similar way as the follicular hormones is taken as further evidence for the view that the follicular hormones themselves can not be considered carcinogenic. Results of experiments carried out with Kaufmann, Müller, and Kröning on the effect of follicular hormones on tumor production in 3,000 mice are referred to in support of the above view. Under section D the virus infection theory is discussed at length. The suggestion is made that the different carcinogens might be thought of as affecting a virus infected cell in such a way as to upset the balance in favor of the virus which would then act to bring about a malignant change in the cell. The genetic influence in carcinogenesis is emphasized and the problem of somatic mutation is mentioned. With regard to the latter, Butenandt refers to negative results of Piepho who attempted to influence the rate of spontaneous mutation in germ cells of *Drosophila* pupae by benzpyrene. The final section on the chemistry of tumors E, briefly treats the work of Warburg on aerobic glycolysis, that of von Euler on the low cytochrome content of tumors and the high ratio of dihydro-cozymase:cozymase in Jensen sarcoma. Butenandt discusses at some length the work of Kögl on the presence of nonnatural glutamic acid in tumor proteins, and briefly refers to the reports of Waldschmidt-Leitz on the presence of d-peptidases in the blood of cancer patients, and the influence of d-peptides on carcinogenesis. Section E is not reviewed critically by Butenandt. Most of the material presented in this section has not been confirmed, particularly that of Kögl and Waldschmidt-Leitz.—P. P. C.

KENNAWAY, E. L. (The Chester Beatty Research Inst., The Roy. Cancer Hosp. (Free), London, England.) Notes on the current literature of cancer research. Cancer Research, 1:164-165. 1941.

A survey of difficulties in making available the data of cancer research. Five types of problems which arise in abstracting are discussed: I. "The manifold aspects of the cancer problem cause the relevant papers to be distributed over a very large number of journals." II. The literature of cancer research would be lightened if the subjects of surgical technic and nursing care could be left to other abstracting journals. III. Too many postmortem reports, especially those including irrelevant details, are published. IV. Papers of importance are often published in journals whose titles do not indicate that such journals contain articles on cancer research. In this way, valuable contributions may escape notice. V. Japanese journals publish an increasing number of articles in Japanese with no summary in any European language.—J. S. L.

COMPARATIVE ONCOLOGY

BURROWS, H. (The Chester Beatty Research Inst., The Roy. Cancer Hosp. (Free), London, England.) Spontaneous uterine and mammary tumours in the rabbit. J. Path. & Bact., 51:385-390. 1940.

In 25 female rabbits which were under observation for more than 900 days, adenocarcinoma of the uterus appeared in 15. Cystic mastopathy was noted in 14. Primary uterine and mammary tumors (the latter multicentric and probably malignant) were present simultaneously in 3 instances. Of the 25 rabbits, 15 had been treated in various ways with 1,2,5,6-dibenzanthracene, and of these 10 had uterine tumors. Of the remaining 10 rabbits which had not received any carcinogenic compound, uterine tumors developed in 5. Hence, it is unlikely that 1,2,5,6-dibenzanthracene played any part in inducing the neoplasia. The ovaries showed advanced changes (enlargement due to intracellular deposition of lipoid) and it is suggested that these may have been concerned in the causation of the tumors. Most of the rabbits had been repeatedly pseudopregnant.—A. H.

CLINICAL AND PATHOLOGICAL REPORTS

Diagnosis—General

BENGOLEA, A. J., and L. P. COSTA. (Rivadavia Hosp., Buenos Aires.) Valor práctico de la punción diagnóstica en los tumores de la mama. Practical value of aspiration biopsy in the tumors of the mammary gland. Rev. méd.-quir. de pat. fem., 16:419-426. 1940.

The author emphasises the simplicity and efficiency of this diagnostic procedure. Four photomicrographs and 2 color drawings are appended.—M. D-R.

Therapy—General

LEE, L. E., JR. (Massachusetts Dept. of Health, Boston, Mass.) Medication in the control of pain in terminal cancer. J. A. M. A., 116:216. 1941.

Emphasis on the use of nonnarcotic medication in preference to narcotics.—H. G. W.

Skin and Subcutaneous Tissue

RIZZOLO, P. J. (Rivadavia Hosp., Buenos Aires.) Sarcoma de las partes blandas subcutáneas. Sarcoma from the subcutaneous tissue. Rev. méd.-quir. de pat. fem., 16:180-185. 1940.

Case report of recurrent leiosarcoma arising from the subcutaneous tissue of the mastoid region.—M. D-R.

Nervous System

SCHWARTZ, C. W. (Neurological Inst., New York, N. Y.) The cranial and intracranial epidermoidomas. Am. J. Roentgenol., 45:18-26. 1941.

A discussion from the roentgenologic viewpoint.—H. G. W.

Breast

ATKINS, H. J. B. (Guy's Hosp., Mastitis Clinic, London, England.) Treatment of chronic mastitis. Lancet, 2:411-413. 1940.

This paper records the influence of estrogens (estradiol benzoate, stilbestrol) and an androgen (testosterone propionate) on the course of chronic mastitis. The effects were estimated clinically and also by the examination of biopsy specimens, special attention being paid to the number of glandular systems (adenosis), the extent of epithelial proliferation (epitheliosis) and the degree of fibroblastic activity. Certain of the histological features of chronic mastitis appeared to be more advanced

after the administration of estrogens. This view corresponded with the clinical findings and suggested that abnormal activity of estrogens might be a factor in the causation of chronic mastitis. Androgens were observed to alleviate the symptoms of most cases of chronic mastitis, but the effect was not permanent or accompanied by any significant histological alterations. In 1 case of chronic mastitis, the only one of 212 to become malignant, carcinoma of the breast developed 10 months after a course of testosterone propionate.—A. H.

Female Genital Tract

ALTHABE, A., G. Di PAOLA, and D. COLILLAS. (Rivadavia Hosp., Buenos Aires.) Arrenoblastoma del ovario. Ovarian arrhenoblastoma. Rev. méd.-quir. de pat. fem., 15: 88-104. 1940.

The tumor was located in the right ovary and was of intermediate type according to Meyer's classification. It induced a pronounced syndrome of virilization and simultaneous defeminization which disappeared after operative removal (except hyperthricosis). The patient became pregnant 10 months after the operation. The paper is illustrated with 9 figures.—M. D-R.

COLILLAS, D., and A. A. DIAZ COLODRERO. (Rivadavia Hosp., Buenos Aires.) Sobre tumores de ovario. Ovarian tumors. Rev. méd.-quir. de pat. fem., 16:427-231. 1940.

Out of 132,000 women treated in Rivadavia Hospital in the last 18 years, 1,059 have been operated on for ovarian tumors (0.80%). The author classifies these tumors in much the same way as the classification of R. Meyer, and gives a brief account of some of the rarer tumors.—M. D-R.

ETCHEVERRY, M. A., and R. MARTINEZ de HOZ. (Rivadavia Hosp., Buenos Aires.) Tumor de Brenner. Brenner's tumor. Rev. méd.-quir. de pat. fem., 16:432-439. 1940.

Review of the work on classification and histopathology of Brenner's tumor of the ovary, and description of a new case. Twelve photomicrographs are appended.—M. D-R.

ZUCKERMANN, C. (Mexico City.) Cáncer peritoneal generalizado de origen ovárico. Pflugerioblastoma. Generalized peritoneal carcinoma of ovarian origin. Rev. de med. y cir. de la Habana, 54:311-317. 1940.

The case was first diagnosed as tuberculosis of the peritoneum. The correct diagnosis was established after laparotomy when primary tumors in both ovaries were discovered. Two photomicrographs are appended.—M. D-R.

FERRANDO, F. F. (Rivadavia Hosp., Buenos Aires.) Las tumoraciones incluidas en el ligamento ancho pelviano. Tumors included in the broad ligament of the uterus. Rev. méd.-quir. de pat. fem., 15:191-204. 1940.

Out of 342 cystic formations of the ovary the author has observed 23 cases of such tumors (7%) which he broadly classifies in 3 groups, as far as treatment is concerned: (a) Unilateral tumors coexisting with normal internal sex organs; in these cases the cyst is simply removed. (b) Same tumors with adhesions to the fallopian tube; in these cases the tube is removed together with the tumor. (c) Large tumors deeply implanted with very firm adhesions; in these cases hysterectomy was performed. Twenty clinical histories are reviewed.—M. D-R.

Urinary System—Male and Female

COOK, E. N., and COUNSELLER, V. S. (Mayo Clinic, Rochester, Minn.) Primary epithelioma of the ureter. J. A. M. A., 116:122-126. 1941.

Clinical study, based on 18 cases.—H. G. W.

O'CONOR, V. J. (Chicago, Ill.) The treatment of malignant tumors of the adult kidney. South. M. J., 34:27-31. 1941.

Clinical study based on 34 nephrectomies for renal tumor, 10 of which were in the renal pelvis.—H. G. W.

Intrathoracic Tumors—Lungs—Pleura

BOHROD, M. G. (Methodist Hosp. of Central Illinois, Peoria, Ill.) Pleural mesothelioma with unusual rate of growth. Am. J. Clin. Path., 11:60-67. 1941.

Report of a case in which the growth was estimated at 27.6 gm. per day.—H. G. W.

Gastrointestinal Tract

FERRARI, R. C., and A. LENTINO. (Rivadavia Hosp., Buenos Aires.) El tratamiento quirúrgico del cáncer del esófago. Revista crítica. Surgical treatment of cancer of the esophagus. A critical review. Rev. méd.-quir. de pat. fem., 14:1224-1267. 1939.

A complete review with 141 references and 29 text figures mostly reproduced from other authors.—M. D-R.

FRENCH, L. R., and GARLAND, L. H. (Stanford Univ. Med. Sch., San Francisco, Calif.) Leiomyosarcoma of the esophagus. Am. J. Roentgenol., 45:27-31. 1941.

Case report with review of the literature.—H. G. W.

HUERGO PINO, M., U. CASTELLANOS, and A. N. NÚÑEZ. (Calixto García Hosp., Habana, Cuba.) Peritonitis por perforación de adenocarcinoma del ileon. Peritonitis following perforation of adenocarcinoma of the ileum. Rev. de med. y cir. de la Habana, 45:233-424. 1940.

The text contains 5 figures, and 9 references of similar cases are appended.—M. D-R.

JONES, T. E. (Cleveland Clinic, Cleveland, Ohio.) Early diagnosis and treatment of carcinoma of the rectum. South. M. J. 34:35-38. 1941.

Clinical discussion.—H. G. W.

MASCIOTTRA, R. L. (Rivadavia Hosp., Buenos Aires.) Carcinoma primitivo del bulbo duodenal. Primary carcinoma of the duodenum. Rev. méd.-quir. de pat. fem., 15:178-190. 1940.

Operation was followed by death in 24 hours. The literature is reviewed and discussed. Four photomicrographs and 1 color plate are appended.—M. D-R.

MASCIOTTRA, R. L., and J. M. PAGÉS. (Rivadavia Hosp., Buenos Aires.) Poliposis intestinal múltiple y carcinoma. Multiple intestinal poliposis and carcinoma. Rev. méd.-quir. de pat. fem., 14:1063-1073. 1939.

Report of 1 case.—M. D-R.

Liver

BERMAN, C. (Univ. of the Witwatersrand, Johannesburg, South Africa.) Primary carcinoma of the liver in the Bantu races of South Africa. South African J. M. Sc., 5:54-72. 1940.

While malignancy in the Bantu may affect every organ primary carcinoma of the liver is extraordinarily frequent (Berman, C., South African J. M. Sc., 1:12. 1935.). The material examined consisted of 270 Bantu cases of primary carcinoma of the liver from the records of the Witwatersrand Native Mine Hospitals (229 cases) and the Johannesburg Non-European Hospital (41 cases) during the 9 years, 1925-1933. The cases which occurred among both males and females were more frequent in males. Among the Bantu it is a disease of young adult life. All cases were found in individuals 40 years of age or under.

Statistics relative to the incidence of primary liver cancer derived from the world's literature were analyzed according to geographical and racial distribution. The statistics are presented in tables and diagrams, and on a map of the Southeastern part of Africa. Primary carcinoma of the liver is a rare disease among all the white-skinned races, irrespective of whether they live in Europe, America, Africa, or elsewhere. The autopsy rate is 0.13% in Europe, and 0.25% in America, and the percentage frequency to all forms of carcinoma is 1.1% in Europe and 2.1% in America. In contrast, primary carcinoma of the liver is a common cancer among all pigmented races, in some of whom it is over 40 times more frequent than in Europeans. The autopsy rate for the Bantu is 1.2% and for all races of Asia, 0.85%. The Javanese register the highest post-mortem rate (1.3%) and the Japanese (1.2%) equal the Bantu. The Chinese follow with 0.9%; the Philippine Islanders register 0.44%, and the British Indians 0.32%.

The percentage frequency of primary carcinoma of the liver to all other forms of carcinoma is 35.3% for the African aborigenes (Bantu and Semi-Bantu) and an average of 14.1% for all the other races of Asia. The percentages for the individual races are: Bantu 37.4, Javanese 36.3, Chinese 36.1. British Indians 28.5, Philippine Islanders 22.2, Semi-Bantu 18.7, and Japanese 7.8. The incidence of primary liver cancer among Eurafricans ("Cape Coloureds") is low.

Primary carcinoma of the liver is a disease in which racial factors appear to play a prominent part. The American Negro still exhibits a distinct predisposition towards this disease, as do also emigrated Chinese. The incidence of this disease among Europeans living in Africa and Asia is as low as it is in the Western countries.—S. B-J.

BERMAN, C. (Univ. of the Witwatersrand, Johannesburg, South Africa.) The clinical features of primary carcinoma of the liver in the Bantu races of South Africa. South African J. M. Sc., 5:92-109. 1940.

A description and classification of the clinical features of 66 cases of primary cancer in Bantu, of which 25 were under the author's own care.—E. L. K.

GILLMAN, J. (Dept. of Anat., Witwatersrand Univ., Johannesburg, South Africa.) Binucleated and multinucleated cells in normal human (Bantu) tissue. South African J. M. Sc., 5:46-53. 1940.

Binucleosis and multinucleosis in man is a frequent accompaniment of pathological lesions (Haythorn, S. Arch. Path., 7:651. 1929). During a recent survey of Bantu tissue Gillman made observations recorded in this paper. Binucleosis in Bantu tissues has additional interest because of high frequency of carcinoma of liver in this people and because in general they appear to be less susceptible to carcinoma than the Europeans. The incidence and distribution of binucleated and multinucleated cells in Bantu, liver, pancreas, stomach, intestine, salivary glands, testis, epididymis, and endocrine glands are described. Binucleosis was found most frequently in stomach, pituitary, and liver. Attention is drawn to the high incidence (20%) of binucleosis in the Bantu liver. The origin of binucleated cells in normal tissue is discussed. The paper is illustrated with 8 photomicrographs.—S. B-J.

PRATES, M. (Inst. Portugais d'Oncologie de Lisbonne, Portugal.) Sur les tumeurs malignes primaires du foie chez les indigènes de la colonie portugaise de Moçambique. Primary malignant tumors (carcinoma) of the liver among the natives of Portugese Mozambique. Presse méd., 47:1550-1553. 1939.

The author summarizes his clinical and anatomical study of 90 cases of primary tumors of the liver among the negro natives of the Portugese colony of Mozambique. The patients came chiefly from the South Sava province, bounded on the north by the territories of Manica and Sofala, and on the south by the frontier of Zululand. Liver cancer appears to be more prevalent in the south than in the north of Mozambique.

These cases of primary tumors of the liver, seen only in negroes, occurred in individuals from 13 to 55 years of age. The average age was under 40. Both sexes were affected; males much more frequently than females. The tumors grew rapidly after first symptoms were noticed. The clinical course is described.

Two types of primary tumors were seen: (1) hepatoma, with various degrees of cirrhosis; (2) carcinoma composed of round or oval small cells in lobulated masses, resembling endothelial cells. In one case the tumor resembled fusocellular sarcoma. The bile passages were free of lesions. Cirrhosis occurred. The tumors were distributed through the liver in masses and nodules, growing by extension and by metastasis within the organ. Pulmonary metastases were rare. No metastases were found in other organs, except in one case in which there was a metastatic growth in the sternum.

Etiological factors of this strictly racial malignant disease localized in regions of Oriental Africa are discussed. Parasitic worm infections probably do not account for the condition. The author calls attention to the large amounts of alcoholic beverages, made from native fruits and plants, consumed by these people. He mentions particularly a distillate of maize (Buputo), a distillate of fermented acajou ("pédoncles de l'acajou"), and a distillate of fermented cassava ("tubercules de manioc"). He believes these beverages may be toxic not only on account of the alcohol in them but because of toxic substances in the barks, fruits, leaves, seeds, etc., from which they are made. The paper is illustrated with 11 photographs.—S. B-J.

Bone and Bone Marrow

MUSCOLO, D. T. (Dept. of Orthopedics, Faculty of Med., Buenos Aires.) Tumores óseos a células gigantes. Giant cell bone tumors. Rev. ortop. y traumatol., 9:301-369. 1940.

General review of the subject and detailed description of 10 cases illustrated by 58 pictures of gross specimens, photomicrographs, and x-ray plates. Treatment consisted of surgical removal of the tumors, or a combination of surgical with x-ray therapy. Patients were followed for from 1 to 2 years after operation.—M. D-R.

PIQUE, J. A., and F. SCHAJOWICZ. (Dept. of Orthopedics, Faculty of Med., Buenos Aires.) Enfermedad de Ollier y encondromatosis múltiples. Ollier's disease and multiple condromatosis. Rev. ortop. y traumatol., 9:303-412. 1940.

Report of 1 case illustrated by 16 figures.—M. D-R.

VALLS, J. E., and F. SCHAJOWICZ. (Dept. of Orthopedics, Faculty of Med., Buenos Aires.) Metástasis óseas simulando tumores primitivos. Bone metastases simulating primary tumors. Rev. ortop. y traumatol., 10:63-82. 1940.

Description of 3 cases of bone metastases of 1 hypernephroma and 2 carcinomas accompanied by 14 good reproductions of photomicrographs and x-ray films.—M. D-R.

Muscle and Tendon

HUTCHINSON, C. W., and D. H. KLING. (Coll. of Med. Evangelists, White Memorial Hosp., Los Angeles, Calif.) Malignant synovioma. Am. J. Cancer, 40:78-84. 1940.

A case of malignant synovioma arising from a bursa at the medial aspect of the knee in a 28 year old subject is described. The author discusses the histological features of synoviomas in general.—L. L. W.

Leukemia, Lymphosarcoma, Hodgkin's Disease

BONNEY, C. W. (Jefferson Med. Coll. Hosp., Philadelphia, Penn.) Primary malignant tumors of the spleen with report of a case of lymphosarcoma. J. Lab. Clin. Med., 26:630-636. 1941.

Case report.—H. G. W.

ISRAËLS, M. C. G. (Dept. of Clin. Investigations and Research, Univ. and Roy. Infirmary, Manchester, England.) The nature of human leukaemia: evidence from the culture of bone marrow cells in vitro. J. Path. & Bact., 51:235. 1940.

Cells from the bone marrow of patients with various forms of leukemia were cultivated *in vitro,* and the developmental potentialities of the cells studied. After about 2 weeks most of the primitive or embryonic cells were converted into cells of a more mature type. The leucocytes lacked recognizable malignant characteristics, such as rapid mitotic division and fixity of type, which have been observed in cells of the transmissible leukemias of animals. The author is therefore in agreement with Ewing's conclusion that the transmissible leukemias of fowls and mice differ from all types of human leukemia.—R. J. L.

MOLINARI, J. L., and A. LEMOS IBAÑEZ. (Rivadavia Hosp., Buenos Aires.) La roentgenterapia de la granulomatosis maligna (Enfermedad de Hogdkin). Roentgentherapy of Hogdkin's disease. Rev. méd.-quir. de pat. fem., 15:105-112. 1940.

A series of 24 cases treated with x-rays with an average survival of 3 years. The authors agree in every respect with the conclusions of Gilbert whose method is followed (V. International Congress of Radiology, Chicago. 1937).—M. D-R.

Thyroid

SYMMERS, D. (Dept. of Hospitals, New York, N. Y.) Congenital Hürthle cell tumor. Arch. Path., 31:99-102. 1941.

The congenital tumor of Hürthle cells, vacuolated cells of either thyroid or parathyroid origin, seems to be unique, as no account of a comparable growth could be found.—H. G. W.

STATISTICS

BERCOVITZ, N. (American Presbyterian Hosp., Hoi How, Hainan, China.) Cancer in Hainan, China. A supplementary statistical study of 451 cases with special reference to age, anatomical distribution, and etiology. Cancer Research, 1: 154-157. 1941.

A comparison is made between the cases of cancer reported in Hainan in 1919 and 1939. Difficulties attending the study of cancer include the lack of records for patients of outpatient clinics and the failure to secure any permissions for autopsies. A large number of cases of cancer is reported in patients under 40 years old, 50.3% in 1919, 41.7% in 1939. In the earlier report, cancer was found in 10 anatomical sites. In 1939, 35 sites are listed. Within recent years certain prejudices have been overcome and gynecological cases and cases of cancer of the stomach have been diagnosed. In 1939, ⅔ of all cases are found to be cancer of the glands of the neck, cancer of the breast, cancer of the skin, and cancer of the penis. The incidence and possible etiology of the various types of cancer is discussed. The incidence of disease in general in Hainan differs in many respects from other parts of the world.—J. S. L.

CRABTREE, J. A. (Nat. Cancer Inst., U. S. Pub. Health Service, Bethesda, Md.) Observations on the familial incidence of cancer. Am. J. Pub. Health, 31:49-56. 1941.

The program of the National Cancer Institute includes the study of cancer from an epidemiological point of view. The epidemiological schedule was designed to provide data for a study of the extent to which factors (occupational, environmental, familial, racial, dietary, economic, medical, and social) may be related etiologically to cancer of various forms and sites. This report presents data from the records of 1,029 cancer patients (522 white females and 507 white males) with reference to the familial incidence of cancer. Although the observations are based on small numbers the following tentative general conclusions are indicated: the incidence of fatal cancer in parents and siblings of white females with cancer of the skin is nearly twice the expected incidence; for patients with cancer of the cervix the familial incidence is more than 1½ times normal; for males with cancer of the skin, excessive familial incidence occurs only for those persons whose skin cancer developed at an early age; for patients with cancer of the lip familial incidence is slightly above normal; for those with cancer of the lung it is considerably below normal. Familial factors involved in the genesis of cancer tend to express themselves early in the life of the individual; cancer of any of the specific sites mentioned (cervix, female breast, skin of males) which develops relatively late in life can more reasonably be ascribed to environmental factors. Cancers of different forms and sites comprise different epidemiological entities. The statistical methods are described and discussed.—S. B-J.

PELLER, S. (Dept. of Anat., Graduate Sch., New York Univ., New York, N. Y.) and C. S. STEPHENSON. (Div. of Preventive Med., Bureau of Med. and Surg., U. S. Navy Dept., Washington, D. C.) Cancer in the mentally ill. Pub. Health Rep., 56:132-149. 1941.

An analysis is given of the 227 cancer cases which occurred in St. Elizabeths Hospital, Washington, D. C., among mental patients during a 10-year period; 189 of the cases were fatal; 155 were examined post-mortem. Expected figures were calculated on the basis of the mortality statistics for New York City (1935).

The distribution of the fatal primary malignancies according to site does not reveal definite deviations from the expectancy. The percentage of skin and lip cancer among the patients is much smaller than expected. Further exploration of this deficit is indicated.

The ratio of cancer deaths for the 10-year period (7.1%) is low; the ratio for the years 1938-40 is higher than for the years 1930-37.

The cancer mortality of the white psychotic patients from the area in which the hospital is located is significantly lower than that of the corresponding white population of New York City. Two-thirds of the patients were born in the South; ⅓ were born in the North, but lived in the South before they were admitted to the hospital. For the group of patients born in the South, and residing there before admission, the mortality was 38.3% less than the expectancy, while for those who were born farther north and who migrated to the South it was 27.0% less than the expectancy.

The cancer mortality of the psychotic ex-soldiers and of the colored mental patients does not deviate from the data calculated on the cancer statistics of the white population of New York City. Of the white patients who were ex-service men, ⅔ were born north of 40° latitude. Only a small part of them was exposed to the climatic conditions of the South either in childhood or later.

The probability of metastasizing to the brain was studied for primary tumors of various organs. Cancer of lungs and adrenals has a higher probability of metastasizing to the cerebrum, exclusive of hypophysis, and cerebellum than cancer of all other organs. Cancer of the prostate has a much higher chance of metastasizing to the hypophysis, the leptomeninges, and the dura.

During the years 1938-40 the number of cancer deaths exceeded the number of tuberculosis deaths among the residents of St. Elizabeths Hospital; previous to 1938 tuberculosis deaths were more numerous.—Authors' summary.

CANCER CONTROL AND PUBLIC HEALTH

PÓLYA, E. (St. Stephen's Hosp., Budapest, Hungary.) The prevention of cancer. Yale J. Biol. & Med., 13:363-385. 1941.

A general discussion of the problem of cancer control based upon theoretical considerations and the author's experience. In the effort to prevent cancer the contributory causes, such as hot or richly spiced foods, should be avoided. Conditions known to be precancerous should be eradicated by medical attention. Intermarriage between members of cancerous families should be prevented. Since cancer may be infectious, discharges from malignant lesions should be prevented from reaching susceptible individuals by segregating the cancerous in hospitals.—A. A. L.